

Twenty years of test ban talk

IN 1958, a conference of scientific experts in Geneva made the first steps towards devising an international seismic monitoring system which would verify compliance with any treaty banning underground nuclear weapons' tests. In 1968, with political interest in a comprehensive test ban in the doldrums but with ten years of seismological research on a national basis completed, SIPRI, the Stockholm International Peace Research Institute, convened further informal meetings of scientists in an attempt to get a comprehensive test ban (CTB) talked about again. Now in 1978, with serious political discussion proceeding both at superpower level and amongst a wide range of nations at the UN Conference of the Committee on Disarmament (CCD), scientists have again reported on what must be done in an international context to monitor a test ban treaty. Their report, the result of deliberations by scientists from 27 countries over a period of a year and a half, has recently been released (as CCD document 558). It reflects substantial credit on its participant, especially on Dr Ulf Ericsson from Sweden, its chairman, and Dr F. Ringdal from Norway, its scientific secretary, for although there was a clear need to hammer out some form of consensus in the document, this has not prevented its message from being clear and unambiguous.

The science of test-ban monitoring was mostly done in the 1960s. Techniques to increase detectability, to discriminate between explosions and earthquakes, to relate seismic magnitude to explosive yield, to locate events more accurately were all developed rapidly during that period, and have in recent years undergone relatively little further change. What has happened in the past ten years, however, has been a marked improvement in data handling. Studies which used to take months of data accumulation and hand measurement can now be done in a morning at a computer console. Many international communication links, both formal and informal, now exist and more are planned. This, of course, is true in many other branches of science and greatly benefits research, but in seismology the bonus is that it is now possible to talk of an international centre or centres, with rapid access to data of a high quality from seismometers all round the world, providing a routine flow of information highly relevant to the verification of a CTB. In many ways the recent report is a blueprint for such an operation, which might be preceded by an experiment taking up to two years.

It is of interest to compare the predictions of network capability which are being made in 1978 with those put

forward in 1968 (which came essentially from a pre-digital era). The detection of events almost invariably depends on the successful registration above noise levels of so-called body waves. This detection capability has improved roughly threefold; explosions of yields of 1 or 2 kilotons in hard rock in most parts of the Northern Hemisphere would now most likely be picked up. The improvement in detection of surface waves, necessary to the identification of explosions as such, is even greater. Identification might now be possible for shots as low as 5 to 10 kilotons in hard rock.

Not all the progress, however, is in the science and technology. For the past twenty years the Soviet Union's willingness to co-operate in a scheme of test-ban monitoring has been in doubt. Many times she has declared that she is perfectly prepared to sign a treaty, but that she regards 'national means' as adequate for verification. Since the Soviet national seismic network is of very limited value in monitoring the United States, this statement is open to the interpretation that the nature of US society is such that clandestine small-yield testing would be impossible. But the corollary is that the nature of Soviet society, and even the geography, leaves the door open to violation and that much wider open if the Soviet Union will provide no data to international agencies. It is too little realised that at present even the informal channels by which seismologists exchange data are closed on the days that the Soviet Union conducts an underground test.

The recent discussions, however, offer some promise. The Soviet Union, a rather hesitant participant to begin with, eventually co-operated fully, and even allowed five of its own stations to be used in various calculations—in contrast to the French and Chinese who stayed away. The next step will be when data from these five stations are supplied on a routine basis. This is unlikely to happen before a treaty is signed—the Soviet Union would regard provision of such material, containing possible evidence of weapons tests, as tantamount to handing out state secrets. But if the long-term intention is to participate fully, this must be regarded as an optimistic sign.

A comprehensive test ban needs much more than a good verification network to bring it into being. But this report is bound to provide some reassurance, particularly in the United States, that such a network, including Soviet stations, is possible. The proposals will not guarantee that tests at the kiloton level can be positively identified as such. But they do show some evidence—for the first time—of truly international goodwill. □



Test failures may hold up space shuttle schedule

MECHANICAL failures during experimental testing of the main engines are threatening to hold up the National Aeronautics and Space Administration's Space Shuttle programme, the first orbital launch of which is scheduled to take place next year.

Three separate test failures have been attributed to problems with turbine blades in the engine's high pressure fuel pump. In two cases the blades were fractured or cracked, and in the third the evidence was destroyed in a fire which followed the malfunction.

Dr Robert A. Frosch, administrator of NASA, said last week that despite the setbacks to the engine testing programme, the agency still had full confidence in the basic design of the engine, and was optimistic that the Space Shuttle programme would proceed broadly on schedule.

However, he admitted that the likelihood of achieving the initially-scheduled March 1979 date for the first manned orbital flight was "considerably less than 50-50", and that the chances of meeting the June deadline to which the agency is now planning were "about two to one in favour".

Dr Frosch's remarks were made in response to a report on the problems of the Space Shuttle engines which had been prepared, at the request of the Senate Committee on Commerce, Science and Transportation, by an ad hoc committee of the National Research Council, under the chairmanship of Professor Eugene E. Covert of the Massachusetts Institute of Technology.

In its report, the NRC committee says that it is confident that the programme will be a success, and that although the development of the engine is not as far along as the timetable might suggest, the problems being encountered were not alarming, but rather typified the early stage of any similar new technological development. And the committee makes various technical suggestions about how some

of the problems might be overcome.

However, the committee adds that achieving acceptable solutions could result in delays to the programme, and that "the ambitious timetable may have to be extended to avoid a first manned orbital flight with a high risk factor."

Furthermore it suggests that some of the problems may have arisen as a result of the heavy pressures under which the Space Shuttle programme is being carried out. In particular these have led to the choice of a "success-orientation", in which every step depends on the success of a number of separate developments each tested in isolation, with little investigation of alternative solutions to particular problems.

Referring to the malfunctioning of the engines during tests, the committee expresses a concern that "the current schedule is so compressed it generates an atmosphere that seems to inhibit realistic evaluations of the problems encountered so far." And it urges NASA to "adhere to currently established testing criteria and standards in spite of the pressures imposed by an ambitious schedule."

The novel and demanding requirements of the Space Shuttle engine—that it should be both capable of propelling an orbital space craft and reusable on a number of successive flights—have necessitated major developments in engine design that the NRC committee recognise as a "prodigious engineering challenge."

The engine provides power by converting liquid hydrogen and liquid oxygen into superheated steam as a propellant. And a major cause for concern has been that during tests the engine has burned at considerably higher temperatures than originally planned.

These higher temperatures are thought to have led to the failure of the turbine blades, which are made of a nickel-based alloy MAR-M-246 originally selected as providing the best

performance at a planned temperature around 1250 °F, rather than the 1500 °F—with brief peaks up to 2100 °F—actually experienced.

NASA is still hoping to be able to reduce the temperature and the vibratory stresses in the engine to a level which will allow such blades to be used. However, Rocketdyne Corporation, which is developing the engine under contract, announced at hearings of the Senate Committee in Washington last Friday that it is now conducting tests on other materials as a precautionary measure.

If it proves necessary to select a new blade material or to make other major design modifications—such as strengthening the shaft and housing—the requisite tests could take up to two years. And this would cause serious delays for the Space Shuttle programme, including the European Space Agency's Spacelab, at present scheduled for launch in 1980.

Such delays would also have embarrassing political consequences for a project whose cost-effectiveness has in the past come in for considerable Congressional criticism, and which President Carter has already tried to cut back by reducing the number of orbiters from five to four.

NASA remains optimistic that the technical difficulties encountered by the engine can be overcome without serious difficulty, and Dr Frosch disputes the NRC committee's criticism of the way that the project is being managed.

However, the agency has agreed to take a long, hard look at the proposed schedule as soon as data is available from the series of engine tests which it intends to carry out over the next few months. And given that its materials' scientists are working at the leading edge of technical knowledge, and that it recognises the fineness of the margin which could separate success from failure, many fingers are remaining tightly crossed.

David Dickson

China's science has 20 years to catch up

CHINA has announced an eight-year crash plan to catch up on the rest of the world in scientific research. Details were given by Vice-Premier Fang Yi to the giant All-Nations Science Conference which opened in the Great Hall of the People, Peking last month.

The 6,000 delegates were told that China was at least 15 to 20 years behind the advanced world in many branches of science and still more in others. The aim was to narrow this gap to 10 years in a number of im-

portant branches by 1985. This would then be a solid foundation for catching up with and overtaking the rest of the world in all branches of science by the end of the century.

Fang Yi was unveiling the draft Outline National Plan for the Development of Science and Technology 1978-1985, which has been drawn up by the recently reconvened State Scientific and Technological Commission. In the next eight years China will increase the number of professional research

workers to 800,000, build research centres with "state of art" facilities and complete a nationwide system of research and development based on these centres.

The plan covers 27 disciplines, gives prominence to eight research fields (see panel) and identifies 108 "key projects".

Fang Yi also opened the door to academic exchanges with other countries. The Chinese, he said, will combine learning from other people

with their own inventiveness. They would like to invite foreign scientists and technical experts to China to give lectures, teach and join them in research.

Earlier, the country's Vice-Chairman, Teng Hsiao-ping, said that China should trust those engaged in scientific work because "generally speaking the overwhelming majority of them are part of the proletariat".

His opening speech to the conference gave full weight to the importance of science and technology. It was tightly bound up with production and was a positive force in advancing society, said Teng. Teng equated dedicated scientists with dedicated revolutionaries and announced China's intention of building "a mighty army of young scientists". This would be done by breaking with convention on the educational front so that selection and training could be achieved in the shortest possible time. Success was possible, he said, because "an extensive mass base can provide a continuous flow of talent". This echoed a statement in a party circular issued last year which said: "Scientific experiments in our country [combine] efforts by both the professionals and the masses. This is something no [western] country has done or can do. Such great mass



Great Hall of the People: planning to overtake the world

movements will open inexhaustible springs of creativity".

Teng promised some relaxation of political control over scientific work. The party would continue to provide leadership but its focus would have to change. One example was the reintroduction of research directors whose positions were downgraded under the rule of the Gang of Four.

The political theme was taken up by Chairman Hua when he addressed the conference. Scientists should continue raising their political consciousness while combining their personal effort with collective wisdom in their research. They would then speak a common language with the workers.

China was training "hundreds of millions of working people" who would combine mental with manual labour, people who would be both worker-intellectuals and intellectual-workers. Hua called for a general rise in the scientific level of the masses which would provide "the base and conditions of growth for the professionals, who for their parts will guide the mass forces, crystallise their experience and wisdom and raise it to a higher level". The world, Hua said, had witnessed different roads to modernisation but what China wanted was socialist modernisation, something which perhaps had not so far been seen.

T. B. Tang

AGRICULTURE. Comprehensive surveys to study the rational exploitation of land resources and the protection of the ecological system. Research devoted to developing a farming system and cultivation techniques which combine both intensive farming and mechanisation. Solutions sought for scientific and technical problems associated with a hydrological project to divert water from the south to the north. Special attention for crop growth on alkaline, lateritic, clay and other types of poor soil, since they account for one-third of the country's total farmland.

ENERGY. Research on gasification, liquefaction, and multi-purpose uses of coal. China is already advanced in petroleum geology and technology; a key project will therefore be investigation of the genesis and distribution of oil and gas, together with the development of new crude oil processing techniques. Other efforts: hydro-electricity generation, solar energy, geothermal, wind, tidal, and thermonuclear fusion power, and energy conservation and the utilisation of wastes in major industrial processes.

Research: China's eight priorities

MATERIALS. Pinpointed areas include metallurgy of special steels, and the refining of copper, aluminium, nickel, cobalt, titanium and vanadium, and the rare-earth metals. Emphasis is on high polymer science, organic synthesis, and catalysis.

COMPUTERS. China aims to put a range of fast main-frames into production shortly and to establish a strong computer network and data base by 1985. Peripherals, software and the associated areas of applied mathematics to receive balanced attention. While speeding up the mass production of LSI's, a special effort will be made to make a breakthrough in the technology of ultra-large-scale integrated circuits. Minicomputers and microprocessors will be introduced.

LASERS. More research in laser spectroscopy and non-linear optics to back up the scientific application of laser. New lasing mechanisms will be investigated, more tunable lasers de-

veloped and applications such as isotope separation fully looked into. High-power installations will be constructed to study laser-triggered fusion. China is also interested in laser communication.

SPACE. Efforts devoted to basic space physics, cosmic rays, and the technical problems of remote sensing. A series of skylabs, deep-space and communication satellites will be launched within eight years.

HIGH-ENERGY PHYSICS. A 50-GeV proton accelerator to be completed in five years and up-dated to still higher energy in the next five. An extensive experimental base will be built around this key research centre to serve agriculture, industry, medicine, and other spheres.

GENETIC ENGINEERING. China is weak in this new field and will spend the first three years mainly on basic studies. By 1985 she hopes to have fully integrated the subject with molecular genetics and cell biology, and be working on its potential use to treat certain diseases and to fix nitrogen biologically.

Harvard scientists split on curriculum reform

THE faculty of Harvard University has been split almost along C. P. Snow's classic "two-cultures" lines over proposals to introduce a core curriculum to be taken by all undergraduates.

Under the new proposals, on which the full faculty is expected to vote at a meeting on 11 April, students would be required to take at least two half courses from each of five areas: literature and the arts, history, social and philosophical analysis, science and mathematics, and foreign languages and culture. There would be additional requirements in expository writing, mathematics and foreign languages.

In aggregate terms, opinion within the faculty on the proposed curriculum seems evenly balanced between those in favour and those against; few are prepared to predict the outcome of the vote. However enthusiastic support from faculty members in the social sciences and humanities has been faced with scepticism from many in the natural sciences.

An informal poll conducted by the *Harvard Crimson*—the university's undergraduate newspaper—revealed that among the former, 55% were in favour of the proposals in their present form, 28% against and 11% undecided, while among natural scientists the situation was the reverse, with almost two-thirds (64%) against the proposals and 22% in favour.

One point of dissent is whether a strict set of requirements is appropriate for Harvard undergraduates. Some students resent the compulsory nature of the proposed core, while staff argue that it could put off the best applicants.

Equally controversial, however, is whether it is educationally possible to provide a significant insight to a non-scientist into the nature of modern science in two courses that may take up only one-sixteenth of an undergraduate's university career and whether this provides an adequate reflection of the role of science in the

modern world.

The core curriculum is the outcome of lengthy discussions that have taken place since the early 1970s under the stimulus of the Dean of Harvard, Dr Henry Rosovsky. In contrast to the general education courses which students have been required to take for the past 30 years, and which aim at developing a broad appreciation of cultural and intellectual issues, the core curriculum as presently proposed attempts to articulate a more structured educational philosophy, its goal being "to have students acquire basic literacy in major forms of intellectual discourse".

A major innovation is that all students would be required to demonstrate an ability in mathematics. A test emphasising algebraic manipulation and quantitative reasoning would be taken at the beginning of their first year, and those who failed would be required to take one of a range of specially-designed courses. Although the compulsory mathematics requirement has raised several objections—some, for example, feel that it could be made part of the entrance requirement—these have been relatively muted. More hotly debated, however, have been the broader aims of the curriculum with respect to the natural sciences.

According to the task force which prepared an outline curriculum submitted to the faculty last month, the aim would be "to convey a general understanding of science as a way of looking at Man and the world". Rather than seeking the breadth of coverage appropriate to departmental introductory courses, the task force suggests that students should "investigate in depth a relatively small number of topics which demonstrate important concepts and methods".

Is teaching science to non-scientists in this way a viable educational goal, given existing constraints on time and resources? Here the science faculties

are split, some claiming it is, while others see it as wishful thinking on the part of non-scientific colleagues.

"How do you offer adequate courses in the physical sciences if people lack sufficient facility with algebra?" asks Professor Frederick H. Abernathy, McKay Professor of Mechanical Engineering. "Perhaps it can be done in isolated cases such as cosmology, but I am afraid that students will end up taking a course that presents physics and chemistry at no more than a rudimentary high-school level".

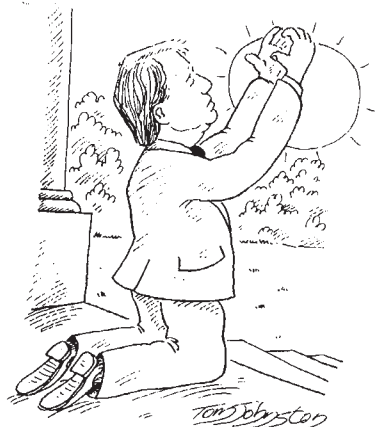
Others are more optimistic. "If you ask whether it is possible to teach science to non-scientists in this way, my answer is yes; it is not only possible but necessary," says Dr David Dressler of the department of biology. "If we don't do it then the chasm between the scientists and the non-scientists will become even wider than it is now. And until one seriously tries to put such an approach into practice, it is wrong to say that it can't and shouldn't be done".

The core curriculum is being enthusiastically backed by the university administration, and in particular by Dean Rosovsky, who has promised substantial resources to put the plan into effect if the faculty passes enabling legislation for him to do so.

But the opposition remains substantial. A straw poll taken three weeks ago at a meeting of faculty professors of the division of applied sciences voted 23 to 3 against approving the proposal, many because they felt its requirements were too rigid; and the physics department is also described as "predominantly opposed" to it.

So next week's debate promises to be a lively one. And even if the new plan is accepted, the success of the core curriculum will depend on the enthusiasm and commitment of those charged with constructing and teaching the courses. As one chemist puts it: "the best taught courses are those which someone is eager to teach". And no-one is disputing that.

David Dickson



"You're the best hope for my energy bill!"

President Carter gives Sun Day official blessing

PRESIDENT Carter, under increasing criticism for his apparent pro-nuclear policies, has given his official support to the designation of May 3 as Sun Day, an idea that originated within a group of environmentalist organisations in Washington, and now promises to involve groups in at least 18 countries throughout the world.

In a presidential proclamation issued last week, the president said that he called upon "the American people to observe that day with appropriate activities and ceremonies that will demonstrate the potential of solar

energy". And he directed all appropriate federal agencies to support such national observance, although adding that success of the federal government's solar energy plans would depend on "an informed and involved public".

According to the Washington-based co-ordinators of Sun Day, requests for information have been received from individuals in more than 60 countries. Specific activities—ranging from academic sessions attended by government ministers in Belgium to a possible ceremony at Stonehenge in Britain—are being scheduled for 1–13 May. □

UK energy forecast: Sussex study sheds doubt on official figures

PRIMARY energy consumption in the UK at the end of the century may be markedly below recent estimates made by Department of Energy officials, according to a report published this week by the Science Policy Research Unit (SPRU) at Sussex University. The difference may lie in a hidden assumption in the Department's calculations (published in Energy Commission Papers 1 and 5) that electricity consumption will remain high at the end of the century, says the Sussex report.

SPRU pays tribute in the report to the "big advance in government thinking" marked by Energy Commission Paper 1, and agrees with the estimates of the DOE (Department of Energy) for 'high' and 'low' growth energy use in the year 2,000. However, SPRU estimates a 25% lower primary fuel consumption for the same low final use as the DOE. "The difference cannot be explained unless a considerably higher level of electricity consumption (and hence conversion losses) was implicitly assumed in Energy Commission Paper 1" says the report.

Electricity is an inefficient way of using primary fossil fuel, involving a conversion efficiency at a modern power station of only 35%. So it tends to be more expensive per useable therm than primary fuel.

Thus it loses ground in market competition with primary fuel—such as North Sea Gas. UK domestic consumption of electricity, for example was about equal to gas consumption in 1960; but by 1976 it was only half, gas having won the greater part of the shrinking domestic solid fuel market. By the end of the century, gas is bound to substitute for non-essential uses of electricity (principally heating) unless government pricing policy artificially prevents it.

The DOE and SPRU appear to agree that the reserves of North Sea Gas and the present contracts with the suppliers all but guarantee a flow of gas of some 6 000 million cubic feet a day at the year 2 000; so the contradiction must lie in the degree to which gas will substitute for electricity. Drs John Surrey and John Chesshire, authors of the SPRU report, argue that if there were to be a low (but quite conventional) economic growth in the UK of 1 to 2% a year, the total energy market would be smaller and the competition fiercer. In this way they reach the conclusion that the bulk of non-essential electricity use would be substituted by gas by the end of the century. There would continue to be substitution even in the high growth (3 to 4), case but it would be less marked.

A relatively low electricity usage at the end of the century is significant for two reasons: first it has a dramatic effect on primary fuel usage through the inefficiency of conventional power stations (which multiplies the effect by roughly three); and second, it affects policy for the near-future commissioning of new power stations. For example, the Department of Energy main forecast allows for 40 GW of installed nuclear capacity by the year 2 000, compared to about 4 GW today. The 25% SPRU variation in primary demand from the DOE 'low' estimate is the electrical equivalent of some 55 GW: sufficient to make a considerable difference in ordering policy.

Drs Surrey and Chesshire were initially very impressed with the DOE forecast contained in Energy Commission Paper 1. The first draft of their study was only five days old when the Energy Commission Paper came out. "We nearly fell out of our chairs" said Dr Surrey this week. "We discussed whether we should bury what we had done. But more and more the very shadowy lower case in the Energy

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Drilling for North Sea Gas: has the Department of Energy underestimated this fuel's competitiveness?

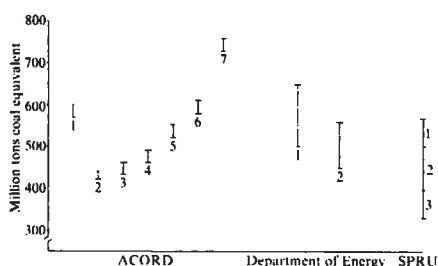
Commission Paper began to worry us". In the report Drs Surrey and Chesshire describe the DOE 'reference' case and then write of the 'less well-documented, almost parenthetical 'lower' case" which is "not sufficiently spelled out".

The 'low' primary consumption in the SPRU forecasts works out at 335 million tons of coal equivalent a year, compared to 450 in the DOE forecast—a difference of 115 mtce. A small part of the difference can be explained by SPRU's lower estimate of the non-energy use of primary fuels, but some 80 to 100 mtce remains to be accounted for. It was here that the SPRU forecasters concluded that the DOE must be making an assumption of high electricity use. "We think such an assumption to be unrealistic" says the report "because under low economic growth, although electricity-specific demands would doubtless continue to expand, there would be no significant substitution by electricity in competitive uses because of the availability of natural gas".

The SPRU report claims to be conservative. "Views are bound to differ about the plausibility of alternative estimates" write the authors. "We put ours forward as a contribution to the debate about future policy options. Like Energy Commission Paper 1, our estimates are based on a fairly 'conventional' view of the future and should not be seen as an extreme 'low growth' scenario involving radical changes in lifestyle".

Robert Walgate

Primary energy demand in the UK in the year 2000: some conflicting forecasts. Left, the 1976 projections of the Department of Energy (DOE) Advisory Committee on Research and Development (ACORD): 1, trends continued; 2, low growth; 3, limit on nuclear; 4, high energy costs; 5, price transition; 6, self-sufficiency; 7, high growth. Centre, the most recent DOE forecasts: 1, Energy Policy Review (1977); 2, Energy Commission Paper 1 (1977). Right, the SPRU results: 1, high economic growth, low conservation; 2, high economic growth, high conservation; 3, low economic growth, high conservation.



The Inca's ancient answer to food shortage

RESEARCHERS in a number of Latin American countries are looking at a crop first cultivated by the ancient Incas and then neglected for centuries, as a possible answer to the Andean region's acute shortage of locally-produced food protein. The crop is quinoa (*Chenopodium quinoa* Willd.), a member of the *Chenopodium* or goose foot family, of which some 60 species are found around the world. Normally sown at altitudes of 2,000 to 4,000 metres, frost-resistant and able to thrive on poor soils with an annual rainfall as low as 300–400mm, its protein content averages about 14% and its lysine content 6%. This makes it superior to most cereal grains in food value. In addition, it contains vitamin C and the B complex of thiamine, riboflavin and niacin.

Peru, Bolivia and Chile are all showing interest in this heritage of Inca agriculture. Bolivia has gone so far as to pass a law requiring the use of at least 5% of quinoa flour in commercially produced breads, pastas and other products. Chileans are using quinoa in feeding programmes to improve the nutrition of poor children. And Peru is trying to increase quinoa production as a means of reducing costly wheat imports.

Quinoa is used in food in a variety of ways, the main uses being in soups and sweets, and a coarse bread called 'kispina'. Various drinks can also be prepared, hot or fermented and high protein cakes and biscuits can be produced by mixing up to 60% quinoa flour with wheat flour. The nutritive value of noodles can also be considerably increased by using up to 40% quinoa flour without affecting the appearance or other characteristics of the end product. Quinoa flakes still retain a slightly bitter taste.

The leaves of the plant can also be eaten in salads and in certain regions where vegetables are scarce the leaves are important. The leaves and stalks are also fed to ruminants, and the chaff and gleanings from threshing are generally fed to pigs.

Along with the potato, quinoa is one of the earliest crops to have been cultivated in the high Andes. It was grown by Indians there long before the arrival of the Europeans and has remained a staple in spite of attempts to introduce European species. Archaeological evidence shows that chenopods were once used in Europe as grain, and various forms of the species are still grown today in hilly areas of north-west India. But the

Andes is the only area where quinoa become an important crop.

The plant is believed to have been domesticated from wild ancestors in highland areas of Bolivia, Colombia and Peru. Unlike potatoes and maize, it was ignored by the Spaniards, and this neglect has continued until recently. The first interest stirred among researchers in Bolivia and Peru in 1965, leading to the development of improved varieties and better



Chenopodium quinoa infructescence

knowledge of the major characteristics of the plant, its cultivation and limitations.

Yields and growing conditions

Yields vary greatly according to growing conditions—from as low as 450 kilograms per hectare to as high as 2,000. The average yield is about 800 to 1,000kg per ha on the altiplano. But a record yield of about 5,000 kg per ha has been reported under ideal conditions near Lake Titicaca, using the new variety *Sajama* developed in Bolivia.

Highland farmers often cultivate quinoa in rotation with other crops because they believe it can prevent disease among other crops. (Quinoa itself is prey principally to mildew and leaf spot, though its most serious enemies are birds).

Tests with rats have shown that quinoa can improve the nutritive value of cereal-based diets. Wheat flour mixed with quinoa flour at a ratio of 4:1 improved nitrogen efficiency for growth by 40%, weight gain by 11%, and protein efficiency ratio by 72% over wheat flour alone.

In reports of poultry feeding trials in Bolivia, chicks fed a ration containing cooked quinoa made gains equal to those receiving corn and skimmed milk. But rations containing uncooked quinoa depressed the rate of weight gain of both chicks and swine.

What makes the difference in growth patterns is the presence of saponins, glucosides that are found in the seedcoat of quinoa. These can be re-

moved by washing or cooking, which removes both the saponins' bitter taste and the toxic effects, which depress growth. In Peru machines have been developed for large-scale processing of quinoa in industry, for example for wheat-quinoa flour mixes. Saponin-free varieties can also be developed, such as the Bolivian variety *Sajama*. Unfortunately, most of the large-grained varieties now in use have a relatively high saponin content.

Research on quinoa

Serious research into the improvement of quinoa began in 1965 at the Patacamaya Research Station in Bolivia, with the support of Oxfam and the United Nations Food and Agricultural Organisation. The station now has a collection of some 700 different ecotypes taken mainly from farmers' fields. Another collection in Puno, Peru, contains some 600 entries, and additional collections are being undertaken. Analysis of these collections is producing an evaluation of the genetic diversity of the plant and could lead to selection of cultivars with desirable characteristics.

The first international convention on chenopods was held at Puno in 1968 and attended only by a small group of researchers, mainly from Bolivia and Peru. A much larger attendance and broader representation was achieved at the second international meeting held in Bolivia in 1975.

Last year, Canada's international Development Research Centre (IDRC) agreed to support an expanded research programme based at the Patacamaya station of the Bolivian Institute of Agricultural Technology. The programme is designed to develop new quinoa varieties adapted to different agro-ecological production zones in Bolivia and elsewhere; develop economic production packages for farmers; and provide training for Bolivian researchers.

Production in Bolivia is currently insufficient to meet the demand created by the law requiring 5% quinoa flour in commercially baked goods, so the research takes on added importance. Research has also been underway at the University of Peru since 1976, supported by the Simon Bolivar Fund and administered by the Inter-American Institute for the Agricultural Sciences.

Edward J. Weber

Illustration from *Plants Consumed by Man* (B. Brouk, Academic Press)

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MIT physicists attack proposed MX missile system

THE new MX missile system, currently under study by the US Defense Department as a possible replacement for the silo-based Minuteman missiles, has been criticised for being too costly, while remaining vulnerable to Soviet attack, by a group of scientists from the Massachusetts Institute of Technology.

The scientists also claim that the MX ("missile experimental") system, which seeks to protect the missiles from attack by placing them in tunnels 10 to 12 miles long in which they can rapidly change firing position, is also likely to create "insuperable arms control problems."

Preliminary studies for the MX system, whose development could cost up to \$30 billion, were begun by the Defense Department in 1974. And the Pentagon has recently informed 10 Western states that they are being screened as possible deployment sites.

Criticism of the proposed system was made in a report published last week by MIT's Program in Science and

Technology for International Security. The authors of the report are Dr Michael B. Callahan, a research associate with PSTIS, Dr Bernard Feld, professor of physics and director of PSTIS, Dr E. Hadjimichael, professor of physics at Fairfield University in Connecticut, and Dr Kosta M. Tsipis, associate director of PSTIS.

The scientists point out that although, until recently, silo-based missiles had been relatively well protected from a surprise Soviet attack, the greater accuracy and explosive power of a new class of ICBM's deployed by the USSR have removed this invulnerability, leading to the MX proposal.

However, the report says that as the MX tunnels would be built to withstand a much lower pressure than the present silos, and would still provide a limited target, a Soviet strike would have little difficulty in destroying the command and control systems.

Furthermore, while accepting that the MX missile would give the US a powerful and accurate intercontinental

ballistic missile, the scientists claim that it would be detrimental to arms control since the uncertainties to which it could give rise might lead the Soviets to adopt a "launch on warning" policy, or even to refuse to accept further limitations on new ICBMs.

As an alternative to the tunnel-based system, the authors suggest that the administration should examine the possibilities for a highly mobile system—perhaps using missiles launched from aircraft or helicopters—which would both permit escape from Soviet attack, and be relatively easy to verify quantitatively by spy satellites.

The Defense Department is already thought to be having second thoughts about the MX system, partly as the result of the technical weaknesses that are being pointed out. Last autumn Defense Secretary Harold Brown decided to delay moving the system into full-scale development, even though Congress has already approved expenditure of up to \$481 million.

David Dickson

Hazards of non-nuclear energy

AT a time when the hazards of nuclear power are under discussion as part of the general debate on energy futures, the UK Health and Safety Commission (HSC) has brought out a report which turns attention to the hazards associated with what it calls the "conventional sources of energy": coal, gas, oil and water.

The report sets out to answer 32 questions put to Tony Benn, the Secretary of State for Energy early last year. The questions came in two letters from representatives of the Staff Associations and Trade Unions at the UK Atomic Energy Authority's (UKAEA) Dounreay Experimental Research Establishment (DERE) in the North of Scotland. A similar report was prepared last year by the Nuclear Installations Inspectorate (NII) of the HSC on 'Some aspects of the safety of nuclear installations in Great Britain'. That report was also initiated by Mr Benn, who asked the NII to answer questions on the health and safety aspects of nuclear power put by himself, Nigel Forman MP and the Friends of the Earth. Many of the questions from DERE are along the same lines as those put to the NII.

The HSC's report attempts to "set the individual questions asked in perspective against the background of the conventional energy industry as a whole". It considers the production and processing of the conventional energy sources up to the point when energy, most commonly in the form of electricity, leaves the producer for

Energy source	operation	Deaths/GWy
Coal	Extraction	1.4
	Transport	0.2
	Generation	0.2
Total		1.8
Oil and gas	Extraction	0.3
	Transport	Insignificant
	Generation	None reported
Total		0.3
Nuclear	Extraction	0.1
	Transport	Insignificant
	Generation and reprocessing	0.15
Total		0.25

Estimated number of deaths due to accidents per GWy (a) of electrical energy sent out

the user.

The HSC found it impossible to identify accidents associated with the transportation of fuel oil to power stations. It has, however, managed to discuss transportation of other fuel including coal, but it is careful to point out that "meaningful statistics are difficult to obtain".

Nevertheless, the report does attempt to discuss many of the hazards of energy production, while warning that its figures are preliminary and should not be taken too literally. The coal-based energy industry poses by far the greatest occupational hazard. UK coal miners suffer many more accidents and are more susceptible to occupational diseases, namely pneumoconiosis, than other energy workers. 64 people were killed in 1975 out of a total workforce of 250,000 in the coal mining

industry. There were 586 serious accidents and 53,898 accidents involving more than three days absence from work.

The transport of liquefied natural gas (LNG), liquefied petroleum gas (LPG) and chlorine (used in power stations) are among the greatest conventional hazards. Because these are all kept under pressure, rupture of the pressure vessel containing them could result in a large release of dangerous gas. In the case of LNG and LPG the danger would be from a massive explosion. In the event of a release of chlorine, the danger would stem from the toxic properties of the gas. Similar accidents, causing as many as 1,000 deaths, could happen if storage tanks failed at a power station.

The report concludes by attempting to make a comparison of the hazards associated with the different energy sources—but it baulks at the task. HSC claims that it cannot compare non-fatal accidents or non-fatal occupational diseases, because of inconsistent reporting procedures. The final, very tentative comparison is therefore made on the basis of fatal accidents resulting from routine operations. The effects of severe rare accidents are not included because "the probability of these events is so small that they would have no significant effect on the long-term averages". On this very tentative basis nuclear power turns out to be the least hazardous energy source.

Judy Redfearn

Recombinant DNA risk-assessment studies to begin at Fort Detrick

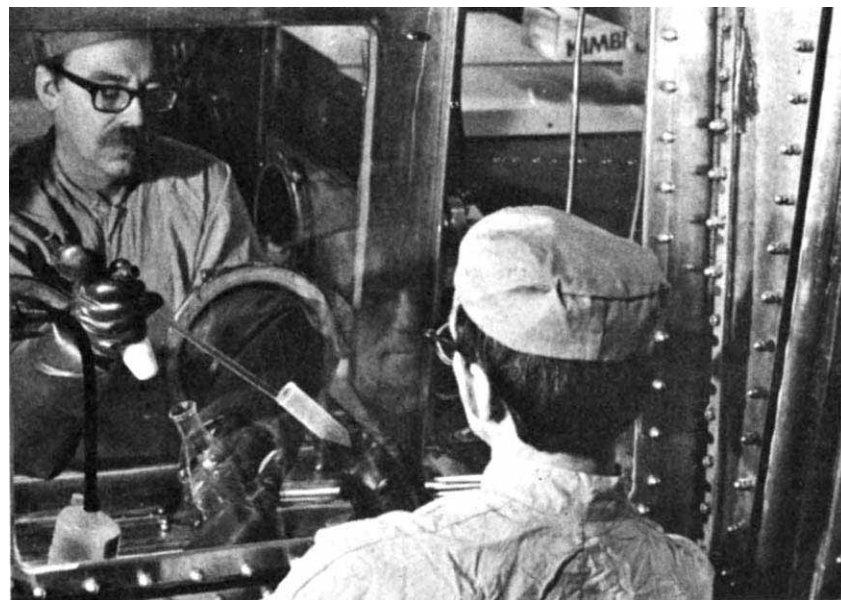
EXPERIMENTS designed to investigate the potential risks of recombinant DNA research are soon to begin in newly-converted, top-level containment laboratories at Fort Detrick, Maryland, until recently the Defense Department's centre for research into chemical and biological weapons.

The experiments will focus on the possible conversion of non-pathogenic bacteria and viruses to virulent forms by insertion of foreign DNA segments, and are being carried out by Dr Wallace Rowe and Dr Malcolm Martin of the National Institute of Allergy and Infectious Diseases.

In particular, DNA taken from mouse polyoma virus will be inserted into *Escherichia coli*, and the bacteria subsequently introduced into mice to see if any neoplasms develop. Such a risk-assessment experiment was first suggested at a meeting in California two years ago by Dr Sidney Brenner of the Medical Research Council's Molecular Biology Laboratory in Cambridge, England.

Similar experiments are already under way at the UK Ministry of Defence's Microbiological Research Establishment at Porton Down. However, the US scientists hope that their greater familiarity with animal experiments will help compensate for an early lead established by their European colleagues.

The opening of the Fort Detrick laboratory—which has been accepted by the National Institutes of Health as meeting the P4 level of physical



Research in progress at Fort Detrick's P-4 facilities

containment—and the beginning of the Rowe-Martin experiment, had been held up by a suit brought by a local lawyer, Mr Ferdinand Mack, on behalf of his 2½-year-old son.

Earlier this month, however, a US District Court judge dismissed Mr Mack's claims that the experiments represented an unacceptable gamble.

Dr Martin said last week that the experiments would begin as soon as a one-week dry-run of the laboratory designed to test its safety precautions had been completed, and that he expected the first results within three to five months. "We have been endeavouring to get this experiment under way for quite a while, and I am looking forward with great eagerness to getting things started," Dr Martin said.

The Fort Detrick laboratory, which

is now run by the Frederick Cancer Research Center and was converted at a cost of \$250,000, is described as an interim facility. Plans have been drawn up for a permanent national P4 facility on the same site which is expected to be in operation in the middle of 1979; other P4 facilities are also being built by the National Institutes of Health in Bethesda.

Dr John Nutter, chief of the NIAID's Office of Specialised Research and Facilities, said last week that the facilities will be reserved for the Rowe-Martin experiments for between four and six months. After this period the laboratory will become available for visitor research, and applications would be invited from other scientists wishing to use the facility, Dr Nutter said.

David Dickson

University patent rights on research: US thinks again

THE US Government has agreed to delay the introduction of a new regulation which would grant universities the patent rights on almost all inventions developed in the course of federally-funded research, a move which opponents claim to be unconstitutional and "contrary to the public interest".

The new rule was published by the General Services Administration in the *Federal Register* at the beginning of February, and was to have come into effect last week. However the GSA has agreed to defer the rule for a period of 120 days at the request of the Office of Management and Budget, itself under pressure from the Senate Subcommittee on Small Businesses.

Under the proposed regulation, universities and non-profit institutions would, subject to certain conditions, be

allowed to retain the entire right, title and interest in patents on inventions made in the course of federally-funded contracts.

Such an arrangement would be covered by an Institutional Patent Agreement of the form which is already applied to federally-funded research carried out in other sectors, and to university research sponsored by the Department of Health Education and Welfare.

The new rules are in line with a recommendation made by the Committee on Intellectual Property and Information of the Federal Co-ordinating Council for Science Engineering and Technology. However, it is being challenged by those who claim that it would lead to a sacrifice by the government of large potential income.

In a letter to the administrator of the GSA, Mr Jay Solomon, consumer advocate Ralph Nader and an associate, Dr Sidney Wolfe, claim the policy to be particularly undesirable in giving away patents whose nature, utility and value are unknown at the time of disposal.

"If this policy is implemented, it is likely that, over the next decade, these institutions will reap hundreds of millions of dollars of profits from work supported by the federal government," the critics claim. "We believe that such a policy is unconstitutional, unwise and contrary to the public interest."

The proposed rules will be the subject of a three-day hearing planned to be held next month by the Senate Committee on Small Businesses, under the chairmanship of Senator Gaylord Nelson.

David Dickson

Atomic scientist to advise India's defence minister

DR RAJA RAMANNA, the director of the Bhabha Atomic Research Centre at Trombay in Bombay, has been appointed scientific adviser to India's Minister of Defence. The appointment appears to have provoked some controversy. Before it was officially confirmed, press reports spoke of Dr Ramanna's "abrupt removal", which a Defence Department spokesman later said was misleading as he had been sounded out some months previously. But *The Times of India* has called it a "wrong decision" and "certainly one of the worst cases of pushing round pegs into square holes". Other reports have described scientists at the Bhabha research centre as gloomy at the news.

The main concern has been over the future of the centre itself, which Dr Ramanna has led for six years. He is regarded as the "father" of India's nuclear capability—it was the Bhabha centre which successfully exploded an underground nuclear device in May 1974 at Pokaran in the western state of Rajasthan, helping to precipitate international concern over the growing trade in nuclear technology. *The Times of India's* fear is that Dr Ramanna's transfer will adversely affect staff morale and that the centre will "recede into the background of routine, uninspired work".

The basis for the suggestion that Dr Ramanna has been a less than willing appointee appears to be that he has been offered the post before and refused it because of his personal wish to remain a working scientist. At the centre he has presided over five research reactors and ancillary plants including a fuel element fabrication facility and a reprocessing plant. Also under his control have been a reactor research centre for fast reactor development in Madras, and a cyclotron in Calcutta.

Nuclear-related policy in India is in the hands of Mr Morarji Desai, the Prime Minister. He cleared the way for resumed co-operation on nuclear research with Canada after the Rajasthan explosion when he gave Mr Pierre Trudeau, the Canadian Prime Minister, assurances last year that there would be no more nuclear tests even for peaceful purposes. The other vexed nuclear issue in India, the resumption of supplies of enriched uranium from the United States for the Tarapur nuclear power station in the State of Maharashtra, now lies in the hands of the US Nuclear Regulatory Commission (NRC), which has to grant the export licence. President Carter has already sanctioned the awaited shipment. □

Agricultural efficiency



KENNETH MELLANBY

As an examiner, I find it difficult to decide how to mark a script when a student includes serious errors in his answer, if it is obvious that he is repeating something he has been taught in his lecture course. Recently I have been marking a question about artificial manures, and all the answers report, rightly, that nitrogenous fertilisers take a lot of energy, generally derived from oil, in their manufacture. The trouble arises when actual figures are quoted, and conclusions are derived from those figures.

I was told by almost all the members of one batch of students that to produce the chemical fertiliser needed to grow one hectare of maize on a modern farm in the United States took 50 tonnes, or 70,000 litres, of oil. This figure is about 250 times too high. Even though this is what they had been taught the figure is so absurd that some marks must be lost. I think that the student should have realised that this amount of oil would cost several thousands of pounds, vastly more than the value of the five tonnes of grain harvested. The most serious result of the overestimate of the energy used to produce the fertiliser is that it gives an entirely false picture of world agriculture, its needs and its potentials.

If we examine the statement that

artificial fertilisers are energy-intensive products, we find that each tonne of nitrogen applied to the soil took approximately 1.8 tonnes of oil equivalent in its manufacture. Intensively grown maize is given approximately 125 kg of nitrogen per hectare. In Britain wheat, producing 4 tonnes per hectare, receives about 100 kg of nitrogen. For the maize this is the energy equivalent of 200 kg of oil, one fifth of a tonne. The figure for wheat is a little lower. Had my students used these data, their answers might have been differently slanted.

If we look at these figures from our own point of view, we see that four tonnes of wheat could supply more than half the calories and much of the protein needed by twenty-four British adults in a year. The nitrogenous fertiliser used to grow one man's ration would be produced by using two gallons of oil—about 50 miles of motoring in a family car.

The richer countries can obviously afford this, but what about the developing countries? We read, and my students repeat in their examination papers, that if the type of farming practiced in Europe and North America were exported worldwide, it would consume 40% of the world's total energy budget. This figure is very misleading, for it is based mainly on a vast increase in intensive animal husbandry. If all farmers used synthetic nitrogen at the UK or USA rate to grow cereals, only something in the region of one hundred million tonnes of oil would be needed for its production. This is a tiny fraction of global consumption.

I would not advocate that all countries followed our example. Both they and we should make better use of our animal excreta and other organic manures, even if producing and spreading them may use more energy than chemical fertilisers. Where there is ample manpower it is foolish to mechanise and produce intolerable unemployment. There is much we can all learn from the traditional agricultural practices of China and other eastern countries. But we must have agricultural policies based on positive concepts and not on nonsensical figures about agricultural energetics. □

Kosmos strikes 1000

KOSMOS-1000, the latest member of the long-running Soviet space series, went into orbit on March 31.

The Kosmos series, inaugurated in March 1962, was originally announced as being for astrophysical/geophysical research. Over the years, its range has gradually been extended "in the service of the national economy". Accordingly,

Kosmos-1000 is "intended for the working out of a space-navigation system, in order to ensure the determination of the location of ships of the naval and fishing fleets at any point of the world ocean".

A review of the Kosmos satellite programme to date will appear in our next issue. □

correspondence

Prenatal screening: information before decision

SIR,—Your editorial about the prenatal screening tests for spina bifida and other congenital deformities (16 February, page 595) is to be welcomed as bringing the often over-heated arguments down onto solid ground. However, your conclusion that "parents will have to realise that a decision to undergo amniocentesis should not be taken unless they are prepared to go through with an induced abortion if the indications are unfavourable" just confirms the suspicions of people like the Archbishop of Glasgow whom you rightly rebuke.

If parents are to be given the chance of making calm and rational decisions then they should have all the available evidence before they make such a decision, rather than after. By saying that because screening is expensive, no foetus which has been found to be deformed should be allowed to survive after birth, parents are being denied the choice of both knowing that a foetus is deformed and of letting it survive.

Screening, as well as allowing a mother to have an abortion, should also be able to prepare, physically and psychologically, the parents of a deformed foetus to become the parents of a living child. Foreknowledge could ease considerably the burden of a family that has agreed to include within itself a congenitally deformed child.

Yours faithfully,

B. DAGNALL

Manchester, UK

Sport throws little light on 'nature v nurture'

SIR,—Professor Bondi's letter on "nature and nurture" (16 March, page 204) is too naïve to let pass. He argues that because sporting success of inhabitants of the DDR has been so outstanding in recent years, as compared with the racially not dissimilar Bundesrepublik, therefore "environment must have a commanding part to play in establishing complex human physical achievements, in spite of the undoubtedly genetic determination of simple physical characteristics, such as eye colour. With such evidence plainly in front of us, what scientific sense can be assigned to attempts to establish the genetic basis of such incredibly complex attributes as 'intelligence'?"

Clearly, whatever may be true of success in competitive sport has no direct bearing on intelligence; even if the argument were meaningful as far as sport is concerned, it has nothing to say about intelligence. If Bondi wishes to address himself to the problem of the genetics of intelligence, he would be well advised to look at the empirical evidence, and to acquaint himself with the theoretical justification for the methods of analysis used; only then would his criticism have any scientific value.

Argument from common-sense analogies is not generally considered to have much scientific standing.

Is anything meaningful being said even about the sporting activities of the DDR? No one has, to my knowledge, suggested that sporting success is 100% determined by heredity; just as no one has suggested anything of the kind for intelligence. We have an estimate of 20% for the environmental contribution to the variance of intelligence; we have no estimate at all for a possible environmental contribution for "success in competitive sport". If this should happen to be in the neighbourhood of 20%, then it would be quite sufficient to account for the observed difference between the DDR and the Bundesrepublik. This would leave 80% of the total variance still accountable in terms of genetic causes.

I do not for a moment take these figures seriously; nothing is in fact known about sporting ability—not even whether in fact there is one, or several, or a large number of such abilities, how they are related, or indeed anything else. We do, however, know quite a lot about intelligence; perhaps Bondi would do well to acquaint himself with this large body of knowledge before taking part in a scientific controversy that passed the level of his contribution eighty years ago.

Yours faithfully,

H. J. EYSENCK

Institute of Psychiatry, London, UK

Athletic Eskimos

SIR,—What Professor Sir Hermann Bondi has shown (16 March, page 204) is that two genetically closely related populations may perform differently under different environmental conditions, as with the training of athletic élites in East and West Germany. But this throws little light on the nature/nurture argument, since even the most hide-bound geneticists don't claim that *all* variation is unaffected by the environment. Sir Hermann has not, of course, shown that inherited factors do not play an important part in athletic performance, nor even that there are no inherited differences in that respect between these two very similar populations.

It may help in illustrating the problems involved to consider the Eskimos from the point of view of sport. They are large, strong, intelligent people, well used to physical exertion, but they have not made much of a name for themselves as yet at basketball, possibly because they have unusually short legs and arms. If the same training and encouragement as is available to athletes in the DDR were to produce a world-beating Eskimo basketball team, this is the sort of evidence needed to support Sir Hermann's thesis that "it follows unambiguously that the environment must have a commanding part to play in establishing complex human achievements", regardless of genetical inheritance. There is however a problem here, which is that resources available for

athletic training, as for everything else, are limited, and some future Eskimo Olympic team might decide to concentrate on other sports, long-distance canoeing perhaps, believing that prospects there were better. Sir Hermann would no doubt consider that a prejudiced attitude, not to say an unscientific one, and it certainly begs the question of the commanding part played by environment in such matters. But it does show the practical difficulties of designing experiments to produce unambiguous answers to the questions he raises, which can seldom be done by looking *post hoc* at particular cases.

Yours faithfully,

C. B. GOODHART

University Museum of Zoology, Cambridge, UK

Training or talent?

SIR,—Professor Bondi's letter (16 March, page 204) suggested that the differences between the athletic success achieved by the DDR and West Germany demonstrated "unambiguously that environment must have a commanding part to play in establishing complex physical achievements".

Professor Bondi would, perhaps, agree that the success of the DDR in fostering athletic achievement is based on the early identification of 'talent' and the provision of every facility for the training and development of that 'talent'.

However, without the 'talent', that is the genetic component, the intensive training and development, that is the environmental component, might not produce results. In this situation how much of the success is due to the existence of talented individuals in the population and how much to training—or is this where we came in?

Yours faithfully,

K. E. REEVE

University of Leicester, UK

Call for engineers

SIR,—The government appointed Committee of Inquiry into the engineering profession, of which I am chairman, is anxious to conduct an analysis of the distribution and deployment of men and women with engineering qualifications, covering both those within engineering functions in industry and those now working in quite different areas of activity.

In this context we are particularly keen to contact people who qualified as engineers but who are now working in fields where their engineering skills are not directly relevant. May I therefore, through the columns of your paper, invite such people who are willing to complete a simple, confidential questionnaire, to send their names and addresses to the Secretariat to the Committee at Ashdown House, 123 Victoria Street, London SW1.

Yours faithfully,

MONTY FINNISTON

news and views

Human reproduction reconsidered

from Robert M. May

EVERYONE has heard of the population bomb, but there is much argument about the length of the fuse and little understanding of how it was lit.

Contrary to some overly simple expositions, the history of human population growth is not one of steady exponential increase. In a seminal review, Deevey (*Sci. Am.* **203**, 195; 1960) identified three phases or 'revolutions'. In the first, or 'cultural revolution', tool-using man emerged as the dominant factor in the evolutionary play. During this roughly 1 million year span, man's numbers remained roughly constant; basing his estimates on the population density for a species making its living by gathering and hunting food, Deevey suggested a very slow rise to a total population of around 5 million toward the end of this time span. Over these hundreds of thousands of years, the average annual population growth rate was of the order of 0.001%. The second phase, or 'agricultural revolution', set in around 10,000 years ago (somewhat later in the New World), and saw men gradually exchange the role of hunter-gatherer for that of farmer. This revolution was accompanied by a qualitative change in population growth rates, resulting in a roughly 20-fold increase in human numbers from around 5 to around 100 million in the period from 10,000 to 5,000 years ago. There followed 5,000 years of relatively slower growth, to around 500 million, up to the dawn of the 'industrial-scientific revolution' some 300 years ago. Since then, the population growth rate has itself increased at an increasing rate: a population of 1,000 million or 1 billion was attained around 1850; the next doubling to 2 billion took roughly 80 years, to around 1930; and today we stand well past the next doubling, at over 4 billion. Many of Deevey's assumptions are necessarily crude, but the broad outlines are reliable.

Keyfitz (*Demography* **3**, 581; 1966) has summarised this in the estimate that the total of number of *Homo sapiens* ever to have lived is approxi-

mately 70 billion. Walt Whitman imagines that 'row upon row rise the phantoms behind us', but, were the ghostly battalions arranged with parade ground precision, only some 20 people would stand behind each human living today. I can think of no more dramatic visualisation of the truly singular nature of our time.

The obverse of this coin is that human populations have been effectively stationary throughout most of their evolutionary history. Accumulating evidence from studies of primitive societies that have (to a debatable degree) survived into the present strongly suggests that the past state of effective zero population growth (ZPG) was not maintained by a Hobbesian balance between brutishly high birth and death rates. Rather, earlier thinking has tended to attribute the past balance to cultural practices that unconsciously serve to diminish fecundity. In the words of Wynne-Edwards (*Animal Dispersion in Relation to Social Behaviours*, Oliver and Boyd, London, 1965): "Man in the paleolithic stage, living as a hunter and gatherer, remained in balance with his natural resources just as other animals do under natural conditions. Generation after generation, his numbers underwent little or no change. Population increase was prevented not by physiological control mechanisms of the kind found in many other mammals but only by behavioural ones, taking the form of traditional customs and taboos. All the stone age tribes that survived into modern times diminished their effective birth rate by at least one of three ritual practices—infanticide, abortion, and abstinence from intercourse. In a few cases, fertility was apparently impaired by surgery during the initiation ceremonies. In many cases, marriage was long deferred". One can think of many practices (for example killing a Bowhead whale to attain true manhood) that may once have served to couple resources with population growth rates.

Recent work, however, indicates that in hunter-gatherer societies, population regulation may have indeed been predominantly by 'physiological control mechanisms of the kind found

in many other mammals'. Drawing much of this work together in a masterly review, Short (*Proc. Roy. Soc. Lond. B.* **195**, 3; 1976) has shown that in primitive hunter-gatherer societies overall fecundity was lower than in almost all present societies due to a combination of later age at menarche and at the onset of fertility, accompanied by long intervals between births caused by suppression of ovulation while breast feeding ('lactational amenorrhoea'). This may have reduced the maximum possible number of children per female to around five, and it does not then require harsh assumptions about mortality rates to end up with ZPG. In this view, the change in nutrition and culture associated with the change from a nomadic to an agrarian way of life led to a breakdown in these physiological control mechanisms, with consequent upsurge in fertility. Higher population densities in settlements, villages, and later towns undoubtedly created circumstances more favourable to the maintenance and transmission of diseases, but on the whole increased fertility outran increased mortality. Short and others suggest that it is mainly at this stage of human evolution that cultural practices became important regulators of population growth. As one example, Saucier (*Current Anthropol.* **13**, 238; 1972) notes that "today long postpartum taboos are particularly associated with primitive communities indulging in extensive agriculture".

Each of these physiological factors—age at menarche, adolescent infertility, lactation and the birth interval—deserves separate attention. The emergent picture forces a reappraisal of many basic assumptions in our society.

Age at menarche

The spectacular decline in the age of first menstruation that has occurred in the western world over the past century was first described by Tanner (*Growth at Adolescence*, Blackwell, Oxford, 1962). Figure 1 shows this. More recent evidence suggests that this downward trend has reached a limit in developed countries, settling

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at a mean age at menarche of 12.4–13.5 years. In many developing countries, the menarchal age is much higher, and probably still declining.

It is reasonable to see this secular trend as correlated with the general fact that children in developing countries have been getting progressively taller and heavier at any given age, presumably due mainly to improved nutrition. In the west, men now reach their maximum height at age 17–18, whereas 50 years ago it was not attained until around age 26. In particular, Frisch and her collaborators have studied the correlation between age at menarche and body weight. They concluded that a minimal level of stored, easily metabolisable energy (in the form of body fat) was necessary for the commencement of menstruation, and assigned a critical body weight of 48 kg at which menarche would typically occur.

As reviewed by Short, these ideas and data remain the subject of debate. Although there are many studies of statistical correlations between age of menarche and nutritional status, there has been little progress in elucidating the specific physiological mechanisms that trigger the onset of puberty. There is, as yet, no easy way of telling when the pubertal waves of ovarian follicular development begin.

Corresponding information about demographic and secular changes in the age of onset of puberty in males is very scant. But this information is somewhat less important in determining a population's net fertility rates; female reproduction is the main rate-limiting process.

Adolescent infertility

In most primitive societies, sexual intercourse begins about the time of puberty and is usually not accompanied by contraceptive precautions. Yet the first pregnancy does not occur for several years. The reason for this adolescent infertility seems to be that ovulation does not typically commence until a few years after the onset of menstruation.

Reviewing anthropological data on the !Kung hunter-gatherers of the Kalahari desert, Kolata (*Science* **185**, 932; 1974) cites 15½ as the average age of menarche and marriage, and 19½ as the average age for producing the first child. In chimpanzees in the wild, 1 to 2.5 years may elapse between menarche and the first conception. Studies based on basal body temperature records of German girls suggest that 60% of menstrual cycles are anovulatory in the 12–14 age group, falling to 45% in the 15–17 group, and 25% in the 18–20 group; maximum fertility is attained around age 26–30 with only 5% cycles anovulatory

Triple overlapping genes

from Maria Szekely

A new sequencing technique and new sequencing results again from Cambridge: this time it is coliphage G4 which illustrates some new tricks a viral genome is capable of in order to compress more information into a small DNA molecule. G4 is a small virus, with a single-stranded circular DNA genome, closely related to Φ X174, the complete primary structure of which has been recently established by Sanger's group (see Sanger *et al.*, *Nature* **265**, 687; 1977; *News and Views* **265**, 685; 1977). Two pairs of overlapping genes have been discovered already in the Φ X174 genome and the same structures exist also in G4 DNA. In addition, however, a third region of overlaps has been found in G4 by Show and coworkers (this issue of *Nature*, page 510). This region shows an even greater efficiency in the use of a single DNA stretch. A tenth viral protein, protein K, which has been detected in relatively large amounts in lysates of G4-infected cells, is coded for by a stretch of DNA which overlaps along its whole length with other genes: its 5' proximal part with the last 86 nucleotides of the gene coding for protein A and its 3' proximal part with the first 89 nucleotides of the gene coding for protein C. At two sites the coding capacity of the DNA is used in all three reading frames. In a TGATG sequence, A is recognised simultaneously as part of the ATG initiation codon of gene K, as part of the TGA termination codon of gene B and as part of the GAT codon for aspartic acid in the sequence of gene A. Similarly in a AAATGAG sequence, the four nucleotides ATGA are recognised in three different reading frames: as part of the codons for Asn and Glu in gene K in the frame AAT GAG; as part of the codon for the last amino acid and the termination codon of gene A in the frame AAA TGA; and as the initiation codon of gene C followed by the beginning of the next, arginine, codon in the frame ATG AGG.

The DNAs of the phages Φ X174 and G4 show a high degree of homology; a gene for a similar protein K could be present in the same position in the Φ X174 genome also. Some genetic evidence has been obtained for the existence of this protein, but it has not been detected

so far in Φ X-infected cells.

These new results have been obtained by a new sequencing technique. Sanger, Nicklen and Coulson recently published a new DNA sequencing method (*Proc. natn. Acad. Sci. U.S.A.* **74**, 5463, 1977), based on similar principles to the plus and minus technique (Sanger & Coulson *J. molec. Biol.* **94**, 441; 1976) which was used in the determination of the nucleotide sequence of Φ X DNA. This new variation is however even quicker, simpler and more accurate than the plus and minus technique.

In the new technique also, a complementary copy is synthesised from a single-stranded DNA template. A random mixture of different length fragments, all ending at a particular base, is obtained using specific chain-terminating inhibitors. These are analogues of nucleoside triphosphates, which have no accessible 3' hydroxyl group. These analogues can be incorporated into the polynucleotide chain through their 5' phosphates but are unable to form further phosphodiester bonds and thus stop further elongation of the chain. By choosing an appropriate ratio of normal nucleoside triphosphate to its chain-terminating analogue, only partial incorporation of the latter will occur and a random mixture of cDNA fragments will be produced which terminate at the different positions at which this particular nucleotide is present. The lengths of the fragments correspond to the different positions of this nucleotide in the DNA sequence.

Four mixtures of fragments can be prepared in the presence of the analogues of the four nucleoside triphosphates. Fractionation according to size will thus reveal the positions of all four nucleotides along the DNA sequence. In this respect these mixtures are similar to the 'minus' mixtures used in the plus and minus technique and the interpretation of the results can be carried out in a similar way. The cDNA fragments in the four mixtures are fractionated by polyacrylamide gel electrophoresis, and from the length of the fragments in the different mixtures, the sequence of the cDNA can be directly read off the gel.

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(Doring *J. Reprod. Fert. Suppl.* **6**, 77; 1969).

Such little evidence as is available

suggests the length of this period of adolescent infertility is roughly independent of the age at menarche. Thus

a drop of, say, 3 years in the menarchal age would (in the absence of any cultural restraints) be accompanied by a drop of 3 years in the age at first pregnancy.

If the changing patterns discussed above, and seen most starkly in Fig. 1, are accepted as real (rather than as biased interpretation of ambiguous data), they have profound social implications. As Short puts it, "In developing countries, with a late age at puberty, the acquisition of fertility and intellectual maturity are almost coincident events which complement one another. In developed countries, on the other hand, it seems that we now acquire our sexuality well in advance of the intellectual maturity that enables us to cope with it. Early teenage pregnancies are something quite new in our evolutionary experience, since hitherto they were a biological impossibility. The social consequences of lowering the age of puberty are probably much more important than the demographic impact of prolonging our reproductive lifespan".

He goes on to declare that "To regard premarital intercourse and contraceptive advice for schoolchildren as stigmata of a decadent, permissive society is to miss the point about the changing biological nature of man".

Conception and pregnancy

In the overall human equation, another parameter that is difficult to determine is the fecundability, defined as the probability of conception per menstrual cycle. A large and varied number of studies suggest figures in the range 20–30% for well nourished women in their 20s. These figures depend on many things, notably age, nutritional status, and the coital frequency (which obviously biases several studies on newlyweds). On the whole, the studies imply a typical 'waiting time' of something like 6–12 months before a menstruating woman becomes pregnant (Potter *Soc. Forces* 54, 36; 1975).

Viewed in this light, humans are a comparatively infertile species. The conception rate in cattle following a single artificial insemination is of order 75%.

Lactational amenorrhoea

The phenomenon of lactational amenorrhoea, whereby ovulation and menstruation are suppressed while the mother is breast-feeding her young, is widespread among the mammals (Frisch *Science* 199, 22; 1978). Since the breast is less efficient at transferring nutrients from mother to offspring than is the placenta, yet has to supply the needs of larger infants, it is easy to see that lactation rather than gestation can become a rate-limiting step in the reproductive process.

As Short says, "the exact physiological processes by which lactation inhibits reproductive activity are poorly understood". Various studies on other mammals suggest that part of the explanation lies in neural impulses from

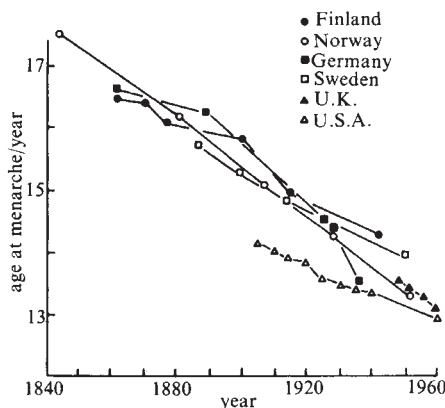


Fig. 1 Secular decline in the age at menarche in developed countries (from Short, *Proc. Roy. Soc. Lond. B.* 195, 3; 1976).

the teat affecting hormonal balances. Thus the duration of lactational amenorrhoea may depend on the frequency of the suckling stimulus, being somewhat shorter if the infant is fed at a few scheduled times each day rather than on demand (Short *op. cit.*). The nutritional state of the mother is also clearly relevant. One of many studies indicative of the metabolic stresses on lactating mothers is due to Rao and Rao (*Indian J. med. Res.* 62, 1619; 1974), who show that pregnant women could easily remain in positive nitrogen balance on an intake of 42 g protein and 2,100 Kcal per day, whereas lactating women show an average negative nitrogen balance of 1 g per day on the same diet. Frisch and MacArthur (*Science* 185, 949; 1974) have indeed suggested that, just as there may be a critical body weight for menarche, so there may be a (somewhat higher) critical weight necessary for the maintenance of menstrual cycles. It follows that in societies where food intake is at the margin, the suppression of ovulation during lactation is probably due to a mixture of nutritional and hormonal factors.

It is hard to know what regulated the birth interval in primitive hunter-gatherer societies. One line of indirect evidence comes from chimpanzees in the wild, where the average interval between successive births is around 6 years (with a range from 4.3 to 7.5 years); this birth interval is shortened by infant death, which suggests it is tied to lactation. Among the nomadic !Kung, the birth interval is about 4 years (Kolata *op. cit.*). Children are breast-fed for 3–4 years, and females are reported to have become pregnant

soon after the death of a nursing infant (Howell *J. hum. Evol.* 5, 25; 1976). If one accepts that postpartum taboos, abortion and infanticide are effectively absent among the nomadic !Kung, the conclusion is that lactational amenorrhoea maintains the 4 year birth interval. Others have contested this view, arguing that the 4 year interval is forced upon nomadic mothers by the exigencies of carting around a child, and suggesting that infanticide follows 15–50% of all births (Dumond *Science* 187, 713; 1974). The !Kung situation is clouded by the uncertainties inherent in anthropological observation, compounded by many of the groups being in various stages of transition between a nomadic, and a more settled agricultural, way of life. For the village-dwelling !Kung, the women are 'fatter', the children are weaned earlier, the birth interval has dropped below 3 years, and the population growth rate has increased by about 30%; we may here be witnessing in microcosm the change in human fertility that characterised the agricultural revolution.

Knodel (*Science* 198, 1111; 1977) has tabulated data on the period of lactational amenorrhoea in three societies that are today in various stages of development: for nursing mothers in Boston in 1963, the average was 5.3 months of postpartum amenorrhoea; for Punjab villages in 1955–59, 10.6 months; for the Havu tribe in Zaire in 1974–75, 17.7 months. The Boston figures agree with data from the North American Hutterites, a well nourished sect whom we will meet again below, where lactational amenorrhoea appears to last for around 5 months. In the absence of breast feeding, postpartum amenorrhoea lasts about 2 months in adequately nourished females.

In summary, Short says "Throughout the world as a whole, more births are prevented by lactation than all other forms of contraception put together." There are today wide-spread trends toward replacing breast feeding by bottle feeding in developing countries, particularly among the urban poor. In his penetrating review, Knodel shows that the net demographic effects of this trend are unclear. On the one hand, the shortening of the interval of postpartum amenorrhoea as the bottle replaces the breast will increase the birth rate. On the other hand, as indicated by a variety of contemporary studies in Third World countries, bottle-fed babies have significantly higher mortality rates. To cite an example, in 15 rural communities in Chile post-neonatal deaths were three times more frequent among infants who started bottle-feeding within their first three months than among those exclusively breast-fed during that time. The reason

MANY tumours produce proteins not normally made in any quantity by the tissue from which the tumour originates. This 'ectopic' production can result in hormones being synthesised in non-endocrine tumours or embryonic proteins being produced in adults. The most common explanation for ectopic protein synthesis is that it is a consequence of derepression of normally inactive genes. While this is a possible explanation in some instances several observations are inconsistent with gene derepression as a general mechanism of ectopic protein production. First, direct measurements using nucleic acid hybridisation have failed to detect significant gene derepression in tumours and second, improved immunological techniques reveal the presence of ectopic proteins in normal tissue (see *News and Views* **269**, 752; 1977). It seems that the most likely explanation for ectopic protein production is the increase in the abundance of mRNAs coding for ectopic proteins rather than new synthesis from previously inactive genes. A number of questions remain; what governs the amount of ectopic protein synthesis, why are certain ectopic proteins made by some tumours and not others and why does ectopic protein synthesis occur anyway?

It has been suggested that the amount of an ectopic protein produced by a cell is a function of its growth rate. There is for instance a positive correlation between α foetoprotein (α FP) production and growth rate in hepatomas; in rapidly growing regenerating liver α FP levels are also elevated (Sell & Becker *J. natn. Cancer. Inst.* **60**, 19; 1978).

Ectopic hormone production by tumours

from Robert Shields

However, the relationship between growth rate and ectopic protein production is not simple, for instance, while human chorionic gonadotropin (hCG) production is high in fast growing and low in slow growing ovarian cystadenocarcinoma cells (Kanabus *et al. Cancer Res.* **38**, 765; 1978) the reverse is true in HeLa cells (Ghosh *et al. Biochem. J.* **166**, 265; 1977). While carcinoembryonic antigen (CEA) secretion is high in patients with inflammatory disease of colon and bronchus it is also high in slow growing bronchial carcinoma cells in culture (Ellison *et al. J. natn. Cancer Inst.* **59**, 309; 1977). These subtle interrelationships between cell growth rate and protein synthesis are not confined to ectopic proteins, the amounts of various proteins made by hepatoma cells can be seen to vary widely under different growth conditions (Ivarie & O'Farrell, *Cell* **13**, 41; 1978). Perhaps it will be possible to make sense of these observations when we have a better understanding of the relationship between cell proliferation and protein synthesis.

Why a specific ectopic protein is often associated with a limited range of tumours and why only certain tumours produce ectopic hormones is not known. Attempts to answer these questions often claim that cancer represents some sort of dedifferentiation to a more embryonic form. For instance, almost all hepatocellular

carcinomas and some pancreatic, gastric and pulmonary adenocarcinomas produce α FP. All these tumours are derived from tissue embryonically related to the yolk sac endoderm which is the embryonic site of α FP synthesis. Dedifferentiation in these tumours is held to result in the re-expression of the ability to make α FP (Sell & Becker *op. cit.*). This dedifferentiation hypothesis is also quite successful in explaining which tumours make CEA. A related embryological explanation for ectopic hormone synthesis is that all endocrine cells and those tumour cells secreting ectopic hormones share a common embryological origin in the neuroectoderm (Pearse & Takor Takor, *Clin. Endocr.* **5**, suppl., 229; 1976). According to this model hormone synthesis by a nonendocrine cell would represent a switch in gene function within a common embryonic lineage. While this idea goes some way towards explaining why some tumours and not others produce ectopic hormones there appear to be some exceptions (Rees *Clin. Endocr.* **5**, suppl., 363; 1976). Of course this idea could be pushed to the ultimate extreme since in the final analysis all tissues in the adult derive from a common precursor, the fertilised egg.

Since so little is known about ectopic protein synthesis it may seem premature to ask why it occurs. There are probably a variety of reasons but a most intriguing possibility is that the production of ectopic protein is of benefit to the cell and has been selected for in the course of the evolution of the tumour. This will be discussed in a future article.

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is simple, particularly in unhygienic surroundings: breast milk is nutritionally ideal, provides some immunity from disease, and is clean. Depending on the exact numerical values assigned to these two countervailing effects, the breast-to-bottle trend could increase or decrease net population growth.

Overall patterns

All the above factors may be drawn together, to arrive at the patterns illustrated in Fig. 2.

For the nomadic !Kung, the relatively late age at menarche, 4 year birth interval, and relatively early age at menopause imply a completed family size of around five (three of whom would typically survive into their reproductive years).

Suppressing the temptation to air Views on their beliefs, I say only that the North American Hutterites are an

Anabaptist sect thought to be reproducing at close to the maximum rate under natural, breast-feeding conditions. The completed family size is around 11, and the population doubling time is a staggering 16 years. Note, however, that the relatively young age of onset of fertility is offset by social restraints that postpone intercourse and marriage until around age 22. Were Hutterites to marry at age 15, there would be a slight increase in the completed family size, but a large increase in the growth rate (which is very sensitive to the age of first reproduction). If they put their minds to it, the Hutterites could possibly double their population every 10 years, thus solving a problem that troubled Sir William Petty in the nineteenth century, when he sought to prove the human population could have doubled every 10 years for a century after the

Biblical flood.

In a splendid review article, Frisch (*op. cit.*) has shown that patterns in mid-nineteenth century Britain were intermediate between the !Kung and the Hutterites. The intermediacy embraces all facets: menarche; end of adolescent infertility; cultural infertility; birth spacing; and menopause. The average completed family size was around 6-8.

Against this background, the patterns (represented schematically in Fig. 2) for today's developed countries are qualitatively different.

Contraception

Short draws a significant message from the facts summarised in Fig. 2: "When we chose to develop artificial forms of contraception, we completely lost sight of the normal reproductive life-history of our species".

If we accept the possible extreme view of the nomadic !Kung encapsulated in Fig. 2, we find that during her reproductive lifespan a primitive hunter-gatherer female would experience 15 years of lactational amenorrhoea, just under 4 years of pregnancy, and just under 4 years of menstrual cycle. In contrast, in developed countries today a female typically experiences menarche at 13 and menopause at 50; two pregnancies with little or no breast-feeding afford about 2 years respite from regular menstrual cycles, which occupy the remaining 35 years of her reproductive life. These possibly exaggerated figures point to a roughly ninefold increase in the time spent having menstrual cycles by females in modern societies, which is something quite outside our previous evolutionary experience.

Any form of contraception that interferes with fertility after ovulation, whether it be male-oriented like the condom or female-oriented like the IUD, will necessarily lead to an increased number of menstrual cycles. But, in Short's words, "It is curious that even with the one really effective form of contraception that acts *before* ovulation, the combined contraceptive pill, we have still chosen to mimic the monthly menstrual rhythm, in the belief that it is more 'normal' than amenorrhoea, and hence more acceptable. More acceptable it may be, but more 'normal' it certainly is not". He goes on to survey the growing evidence that women in western countries would welcome a form of contraception that produced amenorrhoea. It remains true, of course, that many prejudices and

social norms work in the opposite direction, particularly in developing countries.

In addition to a familiar list of symptoms associated with menstruation that women could well do without (not the least, in undeveloped countries, being the blood loss itself), Short argues that our present, evolutionarily new situation may cause other sorts of harm. A variety of epidemiological studies have established that the risk of developing breast cancer is related to the time elapsed from menarche to first birth; the longer the interval, the higher the risk. The data also clearly show that early menopause decreases the breast cancer risk. During the first half of each normal menstrual cycle, the breast is exposed to oestrogen alone, and during the second half of the cycle, to a counterbalancing level of progesterone; it may be safer for the breast to be exposed to an artificially steady oestrogen-progestagen mixture, particularly if oestrogen is the carcinogenic culprit, as has been argued (McMahon, Cole & Brown, *J. natn. Cancer Inst.* **50**, 21; 1973). Short (In *Physiology and Genetics of Reproduction*, (eds Coutinho and Fuchs), Plenum, New York, 1974) has elsewhere suggested that the frequency of other gynaecological disorders, such as carcinoma of the ovary or endometrium, increases as the number of menstrual cycles increases. There is no general agreement on any of these questions, but they obviously deserve careful consideration.

In brief, the process of civilisation and development has, in many subtle ways, affected the natural constraints that held populations in balance

throughout most of the evolutionary history of our species. Many passion-fraught beliefs, on subjects as varied as teenage sex and amenorrhoeal contraception, need to be reconsidered in this light. □

The structural gene for myosin: a closer look

from S. J. Smith

THE humble soil nematode *Caenorhabditis elegans* has proved a useful tool for the extension of the study of gene-protein relationships from prokaryotic to eukaryotic organisms (see *News and Views* **269**, 11; 1977). A variety of uncoordinated (*unc*) mutants can be isolated by their behavioural characteristics, and electron micrographs show that such mutations are associated with disorganisation of muscle structure (Epstein *et al. J. molec. Biol.* **90**, 291; 1974). One such mutant of the *unc-54* gene (e675) has a normal amount of myosin but the number of thick filaments (myosin-containing structures) is much reduced. Characterisation of the mutant myosin showed that it possessed an aberrant heavy chain of molecular weight 2×10^5 daltons rather than the normal 2.1×10^5 component and allowed identification of *unc-54* as the structural gene for a myosin heavy chain representing the bulk of the nematode myosin (MacLeod *et al. J. molec. Biol.* **114**, 133; 1977). More recently, MacLeod *et al. (Proc. natn. Acad. Sci. U.S.A.* **74**, 5336; 1977) have pinpointed the structural alteration of the e675 heavy chain, by following the time course of partial cyanogen bromide digestion of heavy chain fragments labelled specifically at their N-termini; ^{14}C -cyanylation of the wild-type heavy chains generates two large fragments with identical sequences over most of their length, but with different ^{14}C -labelled N-terminal sequences, while cyanylation of the e675 heavy chains produces the same two fragments but shortened by 10^4 daltons. Analysis of the partial cyanogen bromide cleavage products on SDS polyacrylamide gels and by the two-dimensional isoelectric focusing/SDS polyacrylamide gel method of O'Farrell (*J. biol. Chem.* **250**, 4007; 1975) has shown that a cyanogen bromide peptide of molecular weight approximately 4×10^4 daltons can be cleaved from the COOH terminal of the e675 heavy chain without removing

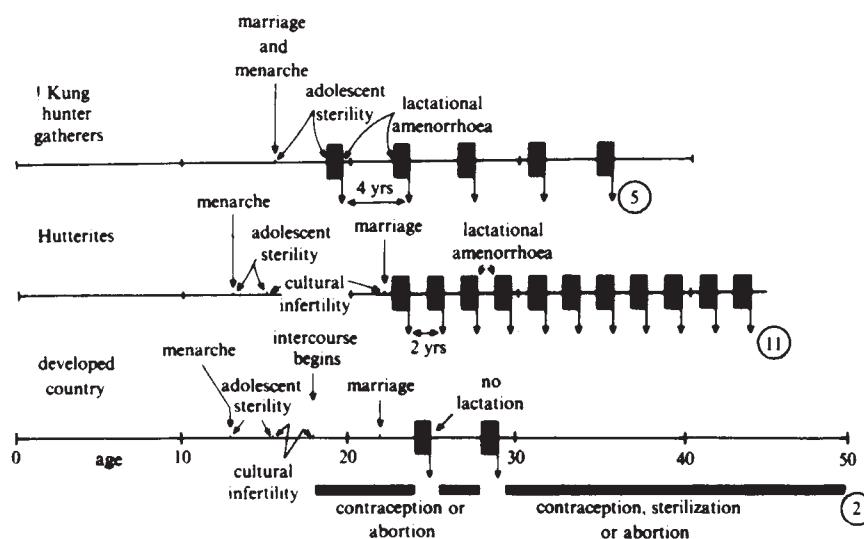


Fig. 2 A schematic representation of changing patterns in human fertility. The nomadic !Kung may approximate primitive hunter-gatherers; the Hutterites are close to the maximum reproductive rate for humans; contemporary patterns in advanced societies are qualitatively different. (From Short *op. cit.*)

the region containing the structural defect. This suggests that e675 is not, as was originally supposed a chain-terminating nonsense mutation, but rather an internal deletion. Its exact position has been assigned because the mutant protein lacks a methionine residue which can give rise to two partial digestion products in the wild-type protein. Successive labelled digestion products are identical showing that the peptide containing the structural defect has been removed. The similarity of the patterns produced by complete cyanogen bromide digestion of the wild-type and mutant proteins indicates that no frame shift on the *unc-54* mRNA has occurred. The identification of the site of the e675 mutation provides a starting point for the fine structure analysis of the *unc-54* gene, while the procedures so far developed should prove useful in mapping mutations in other systems.

Little is known about the regulation and maintenance of the assembly of myofibrillar proteins and the study of *unc* mutants could also lead to a better understanding of the processes involved. MacLeod *et al.* (*J. molec. Biol. op. cit.*) have shown that in addition to the major myosin heavy chain component, which is the product of the *unc-54* gene, there is another type of heavy chain (~ 25% by weight), which is present in both the wild-type and *unc* mutants. This observation suggests that, as in the case of skeletal fast muscle myosin, at least two heavy chain structural genes are expressed. It seems that the different heavy chain gene products will not associate to form hybrid myosin molecules; Schactat *et al.* (*Cell*, **10**, 721; 1977) resolved the e675 myosin into two species homogeneous with respect to the wild-type or mutant heavy chains, while the wild-type myosin itself was not totally homogeneous. An interesting, but as yet unanswered question, is whether thick filaments containing mixed myosin populations can occur. Harris and Epstein (*Cell*, **10**, 709; 1977) have looked at the characteristics of the mutant myosin in an attempt to understand more about the assembly and function of thick filaments. In nematode muscles, as in other invertebrates, the bipolar thick filaments are composed of paramyosin cores surrounded by myosin. The myosin cross-bridges project from the thick filament and are able to bind actin in a polar ATP-sensitive manner. The extreme lability of the nematode proteins has made quantitative comparison of the biochemical properties of the mutant and wild-type proteins impossible, although it has been established that the mutant

e675 myosin forms bipolar filaments, binds actin and has qualitatively similar ATPase characteristics.

Recently mutants with defective paramyosin have been isolated by Waterson *et al.* (*J. molec. Biol.* **117**, 679; 1977) and the structural gene for paramyosin has been identified. In these mutants myosin filaments lacking paramyosin cores are present but these filaments cannot integrate into the myofilament lattice, which is severely disrupted. The presence of large paracrystalline arrays of paramyosin in some of these mutants suggests that the paramyosin-paramyosin interactions may be abnormal although no difference in the mutant protein has been detected in a preliminary screening by SDS polyacrylamide electrophoresis. In one mutant (E1214), paramyosin is totally absent and the body wall structure is disordered; however, in the pharynx, where the myosin is the product of a different gene, organised bipolar thick filament-like structures have been observed. At present it is still unclear whether paramyosin has a purely structural role or whether it has a more specific function such as length determination. Further studies on the interactions of mutant proteins are necessary to explain their failure to assemble correctly and may lead to a better understanding of the normal processes involved in the assembly and maintenance of thick filament structure. □

Sickle-cell haemoglobin fibres

from J. T. Finch

THE haemoglobin molecule is a tetramer of two pairs of identical polypeptide chains, the α and β chains. In sufferers from sickle cell anaemia, a gene mutation has resulted in the replacement of one amino acid, glutamic acid in the sixth position along the β chains, by valine. This replacement while not affecting the solubility of the haemoglobin molecule when oxygenated, drastically reduces the solubility of the deoxygenated haemoglobin so that it precipitates in the cells causing them to become stiff and elongated in a characteristic sickle shape, and impeding their circulation. This elongation strongly suggested that the form of the precipitation is into long aggregates and in fact when sections through deoxygenated sickle cells were examined in the electron microscope, they were found to be filled with long fibrous aggregates running almost the whole length of the cell and wrapping themselves around the inner cell wall

(Döbler & Bertles *J. exp. Med.* **127**, 711; 1968). The fibres appeared very uniform in diameter, measured to be about 180 Å although this might be regarded as a lower limit of the real diameter since the preparation for sectioning requires dehydration and so may possibly involve shrinkage.

An obvious direction for medical treatment of the disease is to administer something to counteract the formation of fibres and to this end a knowledge of the chemical groups involved in the surface contacts between the molecules in the fibres would be of great value. In theory this could be established by an X-ray diffraction study of the fibres in which they are aligned parallel to each other so accurately that the diffraction pattern is not smeared out by the superposition of different orientations. This has proved so difficult that from the X-ray patterns reported to date (Magdoff-Fairchild *et al.* *Nature*, **239**, 217; 1972), it has not even been possible to determine the arrangement of molecules in the fibres unambiguously and it is very unlikely that patterns sufficiently good for a high resolution study could ever be obtained. An X-ray diffraction study of crystals of sickle cell haemoglobin (HbS) is in progress (Wishner *et al.* *J. molec. Biol.* **98**, 179; 1975) but without more knowledge of the fibre structure one cannot be sure that the same molecular contacts occur in both fibre and crystal.

Although one cannot expect to determine the high resolution structure of the fibres by electron microscopy, the study of negatively stained specimens should at least yield the arrangement of molecules. The problem with this method is that deoxy HbS will only aggregate at high concentrations (greater than about 15%) comparable to those within cells. Upon deoxygenation such a solution becomes a viscous gel which needs to be thinned to a fine layer on a support film so that it can be examined in the electron microscope. In our first experiments (Finch *et al.* *Proc. natn. Acad. Sci. U.S.A.* **70**, 718; 1973) the thinning was done by washing the drop of deoxy HbS on the support film with a salt solution and then with stain. The assumption in all this work is that any fibres stuck to the support film remain fixed to it and in the same configuration while being stained and that no new aggregation products are formed by the stain. Although fibrous aggregates were found by this method they were of widely differing diameter, formed by the side-by-side aggregation of varying numbers of linear strings of HbS molecules. The lack of a uniform diameter prompted us to try lysing

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deoxygenated cells directly onto the support film with the hope that by keeping the cell concentration sufficiently low, some of the fibres within them would spill out and become fixed to the support film and remain there in a relatively thin layer suitable for examination after staining. By this means we found collections of fibres of uniform diameter, about 170 Å. The molecules were arranged in rings of six stacked over each other to form a long hollow six-stranded tube. These same fibres were found whether the cells were lysed either by hypotonic salt solution or by phospholipase digestion of the cell membrane.

A similar study was subsequently made by a group in Israel (Josephs *et al.*, *J. molec. Biol.* **102**, 409; 1976), the cells in this case being lysed by the staining solution. While the six-stranded hollow fibres were also found occasionally, the predominant type of fibre in their experiments was not hollow and an eight-stranded structure was proposed in which the molecules in adjacent strands are staggered by about 30 Å. The non-hollow appearance suggests that perhaps other strands were present inside. The overall diameter of these fibres was in the range 180 to 220 Å. This work was continued by Edelstein and his group at Cornell (Crepeau *et al.* *Biochem. biophys. Res. Commun.* **75**, 496; 1977) and more regular versions of the latter type of fibre were prepared from HbS solutions. To facilitate the application of small amounts to the support film, the solution was continuously stirred during deoxygenation to prevent the formation of a thick gel.

Two recent papers have not helped to clarify the situation. In the first of these (Ohtsuki *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 5538; 1977) a group at Chicago essentially reproduce our results in finding six-stranded hollow fibres from cells lysed with water before staining, but also find isolated rings of six molecules in addition to the fibres. The Cornell group on the other hand continue to find the thicker, non-hollow fibres (Dykes *et al.* this issue of *Nature*, page 506) but have reinterpreted their results on the basis of more detailed computational analysis of some of the micrographs and now propose a 14-stranded structure, 10 strands on the outside enclosing 4 inner ones. The 14 strands are approximately hexagonally packed together at any one level with a slightly elliptical overall cross section, but there is a helical twist along the fibre which repeats after about 3,000 Å.

Which then, if any, of these proposed structures represents the *in vivo* fibres? In the original sections through cells, the centre-to-centre distance between fibres was measured as about 180 Å,

even allowing for shrinkage in their preparation it would seem to rule out the 14-strand structure which would have a maximum diameter of about 300 Å. The fibres in the sections do not appear appreciably hollow although stain trapped in a hollow tube could mask this, as could slight tilts of the fibres from the direction of view. The fibres in sections of deoxygenated cells and solutions sometimes appear hexagonally packed and sometimes square packed. The former would favour a six-stranded fibre although one cannot rule out nonspecific close packing. Square packing, on the other hand, appears to be strong evidence for an eight-stranded exterior. However, the method of lysing cells with hypotonic saline would seem to be the mildest and most direct method of preventing the actual structure of the fibres, and with this we have only observed the six-stranded form. It is clear that with a system capable of exhibiting so many polymorphic forms one needs consistent results from material in as near physiological conditions as possible, by a variety of methods of investigation, before one can be convinced that one particular form is the relevant one *in vivo*, and if this should turn out to be a non-hollow tube, there will be a variety of contacts between the surfaces of the molecules and it may not be easy to decide which are those responsible for the precipitation phenomenon. □

Wheat genetics

from F. G. H. Lupton

The Fifth International Wheat Genetics Congress* provided an opportunity for world experts in wheat breeding and genetics to meet workers in a developing country in which wheat yields per hectare have increased by 80% and national production by 180% in the past 12 years. In a keynote address, N. E. Borlaug (CIMMYT, Mexico) stressed the urgency of increasing yields to keep pace with the expansion in world population. Responsibility for this lay partly with the breeders, to provide higher yielding varieties with improved resistance to diseases and pests, but such work must be accompanied by improved crop technology, and in particular by increased use of artificial fertilisers.

The discussions were concerned partly with problems leading to the short term release of improved varieties, and partly with more academic research with long term applications. In the former category, there was considerable discussion on the possible

use of multiline varieties, designed to limit the impact of foliar diseases, and comprising mixtures of closely related and phenotypically similar lines, each carrying different genes for disease resistance. Evidence of delay of development of crown rust (*Puccinia coronata*) in oats in multiline varieties was given by K. J. Frey and J. A. Browning (Iowa State University). This was supported by work on stripe rust (*P. striiformis*) in wheat by L. J. M. Groenewegen (Zelder Plant Breeding Station, Netherlands) and on leaf rust (*P. recondita*) in wheat by M. V. Rao and J. K. Luthra (Indian Agricultural Research Institute, New Delhi). There was, however, considerable debate on the merits of the multiline approach. In particular, it was pointed out that much of the work on the production of multilines had been on an *ad hoc* basis, but that to be fully effective, it was necessary that the components of the mixture should be selected in such a way as to present the maximum barrier to the spread of the pathogen. It was also pointed out by I. A. Watson (University of Sydney) that losses due to stem rust (*P. graminis*) had been successfully controlled in Australia by careful use of relevant resistance genes after analysis of variation occurring in the pathogen.

Another approach to breeding for disease resistance was given by C. N. Law (Plant Breeding Institute, Cambridge), who had used aneuploid techniques to transfer durable resistance to stripe rust, determined by chromosome 5BS/7BS, to advanced lines in the Cambridge breeding programme. He hoped in this way to combine this form of durable resistance with other forms of resistance carried by the receptor lines. The use of alien species in breeding for disease resistance was discussed by F. Dosba and G. Doussinault (INRA, Rennes, France), who described the transfer of resistance to eyespot (*Pseudocercospora herpotrichoides*) from *Aegilops ventricosa* to the bread wheat cultivar Roajon and demonstrated by monosomic analysis that this resistance had been transferred to chromosome 7D.

Breeding methodology received less consideration than at previous congresses, but much controversy remains. J. W. Snape (Plant Breeding Institute, Cambridge) gave data suggesting that genetic linkage of quantitative characters is frequently of minor importance, so that intermating of F_2 plants is unlikely to increase genetic variability. In contrast M. M. Verma (Punjab Agricultural University, India) reported that although little advantage was gained by intermating F_2 plants selected in the same environment, a considerable increase in genetic variance was obtained by intercrossing selections

*New Delhi, 21 February–1 March.

made in environments exposed to contrasting stress conditions.

There was great interest in the suggestion by M. D. Gale (Plant Breeding Institute, Cambridge) that the *Rht* genes widely used in breeding short strawed wheats have a direct effect in increasing yield, possibly associated with their high endogenous gibberellic acid content. As there is a general correlation between straw height and grain yield, he suggested that breeders should select for tall strawed varieties carrying the *Rht* genes—that is for 'tall dwarfs'.

The effect of alien cytoplasm on the phenotype was considered by a number of workers. S. S. Maan (North Dakota State University) reported that the transfer of the *T. aestivum* genome to the cytoplasm of seven *Aegilops* species resulted in fully fertile plants with near normal growth habit, but that a similar transfer of the *T. durum* genome frequently resulted in plants with shrivelled, non viable seed. Using a cluster analysis, K. Tsunewaki (Kyoto University, Japan) grouped the cytoplasm of 26 species of *Triticum* and *Aegilops* into eight or nine major

plasma types, which revealed the phylogenetic origin of the polyploid species. He also reported a promising male fertility-sterility restoration system comprising the cytoplasm of *Aegilops kotschy* or *Ae. variabilis* and a fertility-restoring gene on chromosome 1B of *T. spelta*. The great interest in the effects of introducing alien cytoplasm between species of *Triticum* and *Aegilops* has led to the establishment of an international cooperative programme comprising Maan, Tsunewaki and I. Panayotov from Bulgaria.

A more remarkable example of wide crossing, involving a hybrid of wheat with barley, using wheat as the female parent, was reported by K. W. Shepherd (University of Adelaide, South Australia). Only one of the F₁ hybrid plants obtained had the expected 28 chromosomes; this was repeatedly backcrossed to wheat and monosomic addition lines selected. These were pollinated by tetraploid *Hordeum bulbosum*, and the resultant 22 chromosome haploids treated with colchicine to give disomic addition lines, which closely resembled the wheat parent, though the presence of barley

proteins could be demonstrated by isoenzyme studies. It was suggested that the addition lines might be used for the transfer of physiological characters, such as salt tolerance, from barley to wheat; their possible use to transfer disease resistance was considered undesirable because of the danger of producing wheat varieties susceptible to strains of pathogens which previously only attacked barley.

The Congress also gave an opportunity for the visitors to see some of the trials and selection plots, mainly using traditional pedigree selection procedures, conducted by the Indian Agricultural Research Institute at Delhi and elsewhere. These trials showed the contrasting irrigated and rainfed conditions which must be considered by Indian wheat breeders in the conduct of their work and emphasised the need for agronomic experiments to show how new varieties should best be grown. □

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Pions probe nuclei

from P. E. Hodgson

Two recent papers have shown how pions can be used to probe the finer details of nuclear shapes and sizes. The method is based on a comparison of positive and negative pion scattering in the region of the pion-nucleon (3,3) resonance, that is the resonance with quantum numbers corresponding to an angular momentum 3/2 and an isospin 3/2. The resonant amplitudes of negative pions with neutrons and of positive pions with protons is three times larger than the resonant amplitudes of negative pions with protons and of positive pions with neutrons. By measuring these amplitudes it is thus possible to find out about the relative numbers of neutrons and protons in various parts of the nucleus, and this makes it a powerful tool for the study of nuclear structure.

In the first of these papers, Egger *et al.* at the Swiss Institute of Nuclear Research (*Phys. Rev. Lett.* **39**, 1608; 1977) measured the elastic scattering of 130 MeV positive and negative pions from ⁴⁰Ca and ⁴⁸Ca. They found very marked differences between the cross sections both in magnitude and in the angular positions of the maximum and minimum cross sections. Analysis of this data shows that there is a difference of about 0.3 fm between the radii of the two nuclei, which is

consistent with the density distributions calculated by Negele.

The second experiment uses the scattering of 230 MeV positive and negative pions by ¹⁸O to study the nuclear deformation of that nucleus. Iverson *et al.* at the Anderson Meson Physics Facility (*Phys. Rev. Lett.* **40**, 17; 1978) measured both the elastic and inelastic scattering of the pions and again found significant differences between them. The differences are quite small for the elastic cross sections but are very marked for the inelastic cross sections. These are in the ratio of 1.66 to 1 for the negative to positive pions. This is in contrast to the absence of any differences in similar measurements for ¹²C.

These differences in the inelastic scattering cross sections can be interpreted in terms of the deformation of the wavefunctions of the two neutrons outside the ¹⁸O core compared with that of the core itself. Distorted wave calculations using the collective model of the nucleus show that the observed inelastic scattering cross sections imply either that the deformation length of the neutrons in ¹⁸O is 1.3 times that of the protons, or that the deformation length of the valence neutrons is three times that of the core. The deformation length is defined as the product of the deformation parameter and the equilibrium radius of the deformed potential.

These results are preliminary, and

the data will certainly be subjected to more detailed analysis. It is already clear that the comparison of positive and negative pion scattering, both elastic and inelastic, is likely to prove a powerful method of determining the shapes and sizes of the proton and neutron distributions in nuclei. As the authors of the second paper remark, pions may now be considered as beginning to deliver on their promise as a new and powerful tool in the study of nuclear structure. □

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A hundred years ago

THE correspondent of the *Scotsman* at Ottawa describes a curious phenomenon which occurred in the end of February at Niagara Falls. In the vicinity of Table Rock the river-bed was dry for hundreds of yards towards the centre of the Horse-shoe Falls, whilst the river below the falls was about twenty-four feet below high-water mark. For three days the appearance of the river both above and below the falls led to the idea that the falls would entirely cease for a time.

From *Nature* **17**, 4 April, 454; 1878.

articles

Formation of discrete islands in linear island chains

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Mantle convection studies indicate that large-scale shear beneath plates acts together with the buoyant uplift of the lower density plume material to produce a succession of discrete plumes and consequently island chains such as Hawaii. The spacing of the Hawaiian Islands requires that its low viscosity shear zone is less than 80 km thick.

PLUMES in the Earth's mantle have long been thought to explain the formation of island chains such as Hawaii. It is unclear, however, what mechanism operates within the mantle to produce the discrete islands in these chains. Mantle convection studies indicate that a large-scale shear flow exists beneath the plates and we suggest that this shear and the buoyant uplift of the lower density plume material act together to produce a succession (in time) of discrete plumes. We describe here a simple laboratory experiment designed to test this idea and give a theoretical explanation of the results. We also present a crude calculation of a mantle with a low-viscosity shear zone and conclude that the observed spacing of the Hawaiian Islands requires that such a zone be thin—less than about 80 km thick. A similar conclusion has been reached by many geophysicists for various reasons.

Plume dynamics

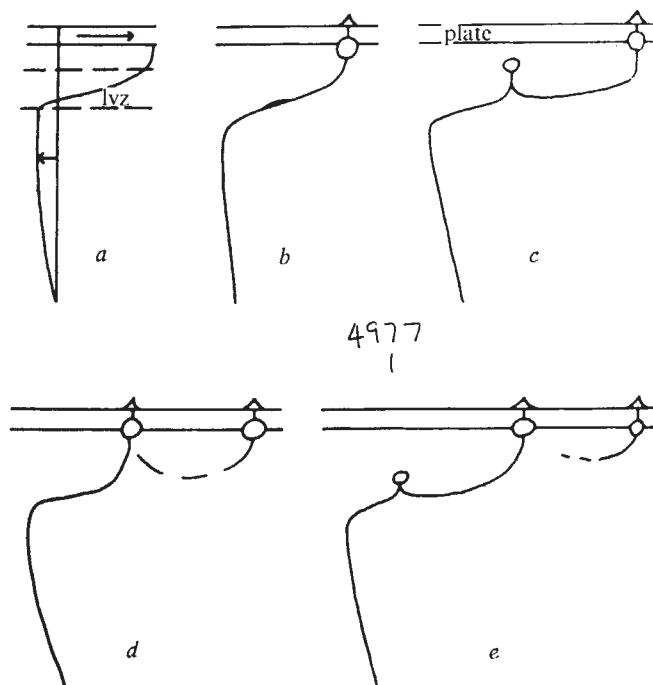
In 1963 Wilson¹ suggested that island chains such as Hawaii were formed as plates passed over a fixed region of mantle where large amounts of magma were being produced. Some years after this suggestion, Morgan² proposed that certain volcanic regions, principally island chains, were the result of plumes of hot rock rising from the core mantle boundary. The fact that these chains aligned themselves with plate motions inferred from seafloor spreading data strengthened the idea. McKenzie and Weiss³ suggested that the upper mantle–lower mantle interface is a more likely source for this material; but also support the idea that such plumes exist within the earth's mantle. Gilluly⁴ has discussed the evolution of magmas associated with the Hawaiian Islands. Each island begins with tholeiitic lava, but changes to alkaline basalt before dying out. This sequence suggests a downward migration of the source with time. His conclusion was that, even though the suggestions of Wilson and Morgan could account for the formation of a chain of islands, it was hard to see why the source of magma should behave in this rise–descend–rise sequence.

Our approach has been to consider a simple problem in plume dynamics and to concentrate on the fluid dynamical aspects of plume behaviour. It has been assumed that there is a source of low density, low-viscosity fluid at some depth in a host fluid of high and possibly variable viscosity which is in a state of shear. In the absence of shear it has been found experimentally that the less viscous fluid pushes out into the host fluid, and its nose is

subjected to a gradually increasing normal stress. At first the fluid forms into a spherical pocket at the source and grows without rising. Eventually the radius becomes large enough so that the sphere rises buoyantly more rapidly than the rate of growth of this radius. The spherical pocket begins to rise when this stage is reached, and as it does so, it trails behind a narrow feeder pipe⁵.

If the host fluid is in a state of shear, it is reasonable to expect that the feeder pipe will deviate from the vertical and assume progressively greater tilt as time increases. As this tilt increases, the buoyancy force causing the fluid to flow along the pipe decreases so that the velocity within the pipe decreases also. If we assume a constant flow rate in the pipe, the radius must increase to conserve mass. It seems inevitable that at sufficiently large angles of tilt, it would be easier for the fluid to form a new nose (possibly aided by the swelling of the pipe) which would then rise vertically, than to move up the small incline of the existing pipe. After some time, this new nose could be expected to form a new spherical pocket trailed by a new vertical feeder pipe which would itself assume progressively greater tilt as time increases. The repetition of such a procedure might lead to the formation of a succession of new blobs. This is illustrated in Fig. 1.

Fig. 1 Schematic diagram showing a possible mode of formation of island chains as the result of the interaction between a low-viscosity pipe, which is rising due to buoyancy, and a horizontal shear flow. *a*, Possible shear flow for mantle with a low viscosity zone (lvz); *b*, formation of a new nose; *c*, a new pipe, and *d*, a new island; *e*, process repeats.



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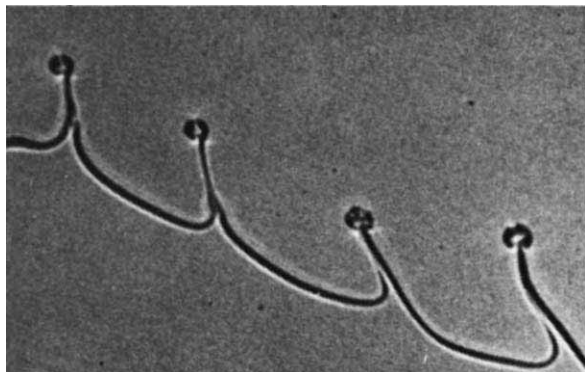


Fig. 2 New pipes formed as a result of an unstable wavelike disturbance on a pipe inclined at about 25° to the horizontal.

By understanding the relationship between the spacing of these blobs and such parameters as the mass flux of the low viscosity fluid, the viscosities of the two fluids, the variation of viscosity with depth in the host fluid, the rate of shear in the host fluid and the density difference between the two fluids, we can apply this concept to real island chains.

Preliminary experiments

Experimentally, a silicon oil of viscosity 10 centistokes (cst) and density 0.934 g cm^{-3} was fed through a pipe into the base of a tank containing oil of viscosity 1,000 cst and density 0.971 g cm^{-3} and allowed to flow until the spherical pocket of fluid had reached the upper surface. A mature plume was thus formed. When this state had been reached, the tank was tilted (causing a minimum of disturbance to the fluids) so that the feeder pipe was inclined. Figure 2 shows the behaviour of a pipe which was tilted to an angle of about 25° to the horizontal. Before tilting, flow in the pipe was laminar and the radius was independent of height; soon after tilting, however, a wave-like disturbance appeared on the pipe. The amplitude of this disturbance increased with time until the pipe took on the form shown in Fig. 2 about 2 min after tilting. This behaviour was only observed in experiments in which the inclination of the pipe to the horizontal was less than 35° . For greater angles of inclination, the pipe appeared to be stable.

Theoretical calculations

A simple theoretical explanation of this behaviour can be derived as follows. We may approximate the uplift velocity law by the expression for the terminal velocity of rods rising or falling through a viscous fluid. The rise velocity of an element of pipe inclined at angle θ to the horizontal is then⁶:

$$W(\theta) = \ln\left(\frac{l}{c}\right) \frac{gc^2\Delta\rho}{8\mu} (3 - \cos 2\theta)$$

where $\Delta\rho$ is the density contrast, μ is the viscosity of the host fluid and l and c are the length and radius of the element of pipe. (In fact, $\ln l/c$ does not vary rapidly with large l/c and a value of 10 for this factor is taken as reasonable.)

We have already indicated that the radius, c , of the pipe also depends on the inclination θ . Whitehead and Luther⁵ have given an expression for c when the pipe is vertical and the buoyancy force along the pipe is proportional to $g\Delta\rho$. When the pipe is inclined, the buoyancy force driving flow along the pipe is proportional to $g \sin \theta$ so that their expression can be accordingly modified. From this we find that

$$c^2 \propto (\sin \theta)^{-1/2}$$

and

$$W(\theta) \propto (3 - \cos 2\theta)/(\sin \theta)^{1/2}$$

This function has a minimum at $\theta_c = 35.3^\circ$. When $\pi/2 > \theta > \theta_c$, $dW/d\theta$ is small and positive; however, when $0 < \theta < \theta_c$, $dW/d\theta$ is

negative and $dW/d\theta \rightarrow -\infty$ as $\theta \rightarrow 0$. Thus, when the inclination of the pipe is less than this critical value, θ_c , small wave-like disturbances will be amplified because the curvature they produce gives rise to a large differential uplift. When $\theta > \theta_c$, however, these disturbances will not grow.

The above considerations have dealt with pipes subject to buoyant uplift but no horizontal shear flow. To study the case in which both factors are present, we have developed a numerical scheme which allows us to trace the evolution of a pipe subject to a specified shear flow, $U(z)$, and uplift $W(\theta)$. From such calculations, in which we specify both velocity functions, we can determine the time necessary for a new nose to be initiated. It is, of course, the inverse problem that we would like to solve for real island chains: given the time between successive islands, can we determine the velocity and hence the viscosity structure of the underlying mantle. We will present more details of these studies elsewhere.

We will consider at present a simple analytical model which allows us to determine the time taken for new nose to form as a function of the other parameters of the problem. To obtain such an analytical relationship, we consider a viscosity of the form

$$\mu = 10^{23} (1 + z^2/L^2)/(1 + h^2/L^2) \text{ poise.}$$

Here z is the vertical displacement from the viscosity minimum, h is the height of the surface (at which we assume the viscosity is prescribed to be 10^{23} poise) and L is the length scale for the variation of μ . (Cgs units are used in all calculations.) Such a viscosity structure leads to a horizontal shear flow of the form

$$U(z) = U_T \tan^{-1}(z/L)/\tan^{-1}(h/L)$$

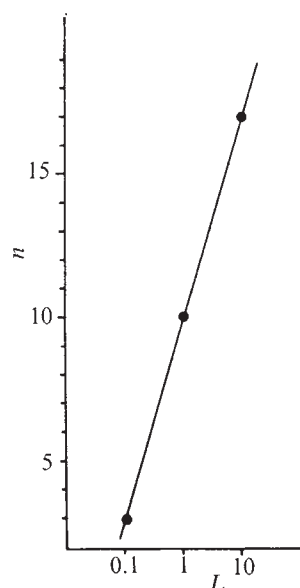
where U_T is the velocity at the surface $z = h$. The full expression for the uplift velocity in this case is found to be

$$W = 10^{-23} \ln\left(\frac{l}{c}\right) \frac{(1 + h^2/L^2)}{(1 + z^2/L^2)} (g\Delta\rho Q \bar{\mu}/8\pi)^{1/2} \frac{(3 - \cos 2\theta)}{(\sin \theta)^{1/2}}$$

where Q is the mass flux of fluid of viscosity $\bar{\mu}$ up the pipe.

We calculate the time required for a new nose to form by a simple two-step procedure. Starting with a vertical pipe, we subject it to shear flow only for time ηt_c , where t_c is the required time. Then we subject the tilted pipe to buoyant uplift only for the remaining time $(1 - \eta) t_c$. t_c is minimised when $\eta = 1/3$. For this second step, we calculate the vertical rise of the pipe at $z = 0$ and at one other value of $z (> 0)$ and require that the difference be

Fig. 3 Variation of L with $\bar{\mu} = 7.4 \times 10^9$ when t_c is taken as 1 Myr.



equal to z . This gives an estimate of the time required to attain a horizontal gradient on the pipe as a function of z . This function has a minimum when $z^2 \simeq 0.85 L^2$. After algebraic manipulation, we find that the minimum value of t_c is given by

$$\ln(h/c) h^2 10^{-23} (gQ\Delta\rho\bar{\mu} U_T t_c^3/8\pi L^7 \tan^{-1} h/L)^{1/2} = 2$$

where we have assumed that $(h/L)^2 \gg 1$. If we consider the Hawaiian chain and estimate $h \simeq 100$ km, $Q \simeq 10^6$ cm³ s⁻¹, $\Delta\rho \simeq 0.25$ g cm⁻³, and $U_T = 3 \times 10^{-7}$ cm s⁻¹ (10 cm yr⁻¹), we find that

$$t_c^3 \bar{\mu}/L^7 = 2 \times 10^{16}$$

If the islands in this chain are approximately 1 Myr apart, we may use this expression to relate the remaining unknowns: L and $\bar{\mu}$. We find that $L \simeq 2,800 \bar{\mu}^{1/7}$ so that L is very insensitive to the value of $\bar{\mu}$ chosen. This is fortunate as there is considerable uncertainty in the value of this parameter. Figure 3 shows that values of L in the range 0.1–10 km are indicated for reasonable values of $\bar{\mu}$. This corresponds to a range of mantle viscosity contrasts of 10^6 – 10^2 and of 90%-shear zone widths of ~ 1 –80 km. Computer calculations using the parameter values given above indicate that the above expressions overestimate t_c by a factor of about 2. Thus the values of L given above are also discrepant but only by a factor of $2^{3/7}$ or about 1.35.

Conclusions

We cannot defend in detail the numbers we have chosen in the above calculation. However, because L is raised to the seventh power in the expression for t_c , the values of L which we predict are insensitive to the other parameters of the problem. We propose, therefore, that the existence of a narrow shear zone in the mantle is necessary for the formation of new islands on the timescale observed at the surface of the earth. This, however, is not a new suggestion; many geophysicists^{7–12} have proposed the existence of such a low viscosity shear zone for a variety of different reasons. Thus, the predictions of our model fit well into an existing conceptual framework which strongly indicates that this mechanism may well be responsible for the formation of discrete islands in island chains.

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Transmission of hormonal stimulation by cell-to-cell communication

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Rat ovarian granulosa cells and mouse myocardial cells respond to cell-specific hormones by cyclic AMP-dependent mechanisms. In coculture, these heterologous cells communicate by means of gap junctions. Exposure of the cocultures to a hormone specific for one cell type causes the heterologous cells to respond through a cell contact-dependent mechanism. These studies suggest that this cross-stimulation results from the intercellular communication of a mediator that is common to both cell types. The communicated mediator may be cyclic AMP.

IN multicellular organisms, cells communicate by various mechanisms, including the formation of low-resistance junctions. Since the discovery of low-resistance connections between neurones¹, this form of cell-to-cell communication has been documented widely in both excitable and nonexcitable cells. These junctions have been correlated with the capacity for intercellular transfer of fluorescent dyes^{2–4}, labelled cations⁵, nucleotides⁶, amino acids⁷ and other metabolites⁸. These and other studies indicate that molecules with a molecular weight of less than approximately 1,200 can pass from cell to cell⁹. Low-resistance pathways (ionic coupling) and nucleotide transfer (metabolic coupling) have also been associated with the presence of a specific cell structure, the gap junction¹⁰. It is generally accepted that gap junctions can provide a structural pathway for the transfer of small molecules from cell to cell¹¹.

Cell communication in the form of ionic coupling allows synchronous electrical activity in the heart, smooth muscle and

some neurones¹². However, in nonexcitable tissues, the function of ionic coupling is unclear because there is no obvious requirement for electrical synchrony. It has been proposed that intercellular channels could allow the cell-to-cell transfer of small molecules that might regulate and synchronise activities such as metabolism, growth and differentiation in multicellular systems^{12–16}.

The studies described in this report were designed to investigate the potential role of communication in hormonally responsive systems. For example, when a hormone stimulates a cell within a target tissue, coordination could be achieved by the gap junctional transfer of low-molecular weight mediators of the response to other cells within the population. This presumptive mechanism is particularly attractive in the case of hormones with effects mediated by cyclic nucleotides. In this regard, there is experimental evidence that cyclic AMP can be transferred between cells because it has been shown that exogenously applied cyclic AMP diffuses through cardiac ventricular muscle, presumably through a gap junctional pathway¹⁷.

To examine this hypothetical mechanism we used a system composed of cultured rat ovarian granulosa cells and mouse myocardial cells, two cell types that communicate through gap junctions and respond to specific, but different hormones by cyclic AMP-dependent mechanisms. If these heterologous cells were able to communicate in culture, it should be possible to determine whether stimulation of one cell type influences the activity of the other. A positive result would suggest that a signal induced by the hormone can be transferred from the stimulated cell to the heterologous cell which is unresponsive to that hormone when cultured alone. We report here evidence that cross stimulation does occur in communicating cocultures of granulosa and myocardial cells.

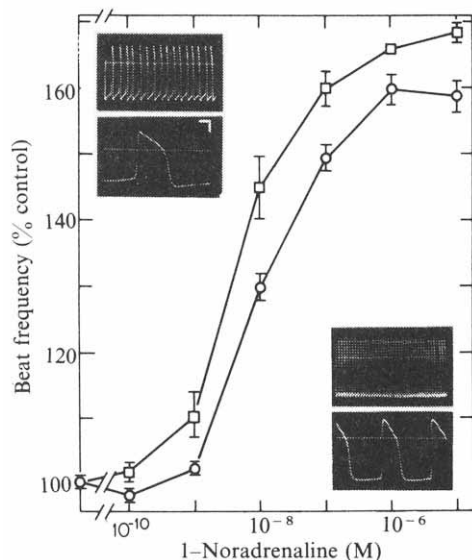


Fig. 1 Effect of 1-noradrenaline on beat frequency and action potential of cultured myocardial cells. Cells were prepared according to Epstein and Gilula³⁸. For beat frequency experiments, 5×10^4 – 1×10^5 cells were plated into Falcon multiwell plates (3008) in 0.5 ml of Eagle's minimum essential medium (MEM) without glutamine. After 2–4 d in culture and at least 3 h before beginning an experiment, the medium was changed to Leibovitz's L-15 (phosphate-buffered medium that remains near pH 7.4 in air). Both L-15 and MEM were supplemented with 10% calf serum, penicillin (100 U ml^{-1}) and streptomycin ($100 \mu\text{g ml}^{-1}$). All media components were purchased from Gibco. □, Cultures were preincubated for 20 min in $3 \times 10^{-6} \text{ M}$ MIX (Calbiochem), while the other cultures (○) were incubated without MIX. Noradrenaline-HCl (Sigma) was added in $10\text{-}\mu\text{l}$ samples and beat frequency was counted 10 min later. Cultures were kept in a 37°C room during the experiment. Each value reported is the mean $\pm$ s.e. (○, $n = 6$; □, $n = 3$). For electrophysiology, the cells were plated into chambers constructed of stainless steel rings attached to 35-mm Falcon dishes with silicone grease (Dow Corning) ($1\text{--}2 \times 10^5$ cells per ring). The medium and rings were removed 18 h later and L-15 with 10% calf serum was added to the dish. During experiments the culture medium was maintained at $37^\circ\text{C} \pm 0.5$ by a heated microscope stage. The microelectrodes were prepared from self-filling capillary tubing (Hilgenberg) and had resistances of 40–100 M Ω when filled with 3 M KCl. The electrophysiological equipment and methods have been described before³⁸. Records were obtained by continuous impalement of one cell so that each cell served as its own control. Oscilloscope traces in the upper left were recorded before treatment of cultures with $2 \times 10^{-6} \text{ M}$ noradrenaline. Those in the lower right were obtained approximately 10 min after addition of noradrenaline. The vertical calibration bar is 20 mV and the horizontal bar is 2 s for each top trace and 100 ms for each bottom trace. The horizontal line represents the bath potential.

Characterisation of myocardial and granulosa cell responses

In the mammalian myocardium, the effects of catecholamines seem to be mediated by cyclic AMP^{18–21}, and this also seems to be the case in cultured rat or mouse myocardial cells. It is known that catecholamines cause an increase in intracellular cyclic AMP levels^{22,23} and that catecholamines 24,25 and cyclic AMP analogues^{26,27} increase the spontaneous beat frequency of these cells. The electrophysiological studies described here document this result, and demonstrate an increase in the amplitude of the action potential and a decrease in the action potential duration. Compared with control values, treatment of the cultures with $2 \times 10^{-6} \text{ M}$ noradrenaline resulted in a beat frequency of $181 \pm 13\%$, an action potential amplitude of $110 \pm 1\%$, and an action potential duration of $72 \pm 3\%$ ($n = 10$) (Fig. 1). Addition of 1 mM dibutylcyclic AMP (Bt₂ cyclic AMP) changed the beat frequency to $142 \pm 6\%$, the action potential amplitude to $111 \pm 1\%$ and the action potential duration to $81 \pm 2\%$ ($n = 9$) (Fig. 2). In identical conditions, 1 mM butyric acid had no effect on these parameters. (Unless otherwise indicated, averages are presented

as the mean $\pm$ the standard error compared with control values.)

Increases in beat frequency also resulted from the exposure of cultures to inhibitors of cyclic nucleotide phosphodiesterase. Because theophylline gave variable results in the range of 1 mM the more potent inhibitor 1-methyl-3-isobutylxanthine (MIX) was used routinely. MIX caused an increase in beat frequency with a half maximal response at $1\text{--}3 \times 10^{-5} \text{ M}$ and a plateau at $3 \times 10^{-4} \text{ M}$. The maximal response with this drug was 50% above control. In this concentration range, MIX also potentiated the effects of noradrenaline and Bt₂-cyclic AMP on beat frequency (Figs 1 and 2).

Cultured rat ovarian granulosa cells respond to follicle stimulating hormone (FSH) by producing plasminogen activator^{28,29}. The following findings suggest that this response is mediated by cyclic AMP. (1) The effect of FSH is potentiated by cyclic nucleotide phosphodiesterase inhibitors²⁸; (2) the effects of FSH are mimicked by Bt₂-cyclic AMP^{28,30}, and (3) FSH causes a rapid, dose-dependent increase in granulosa cell cyclic AMP levels³¹ that is similar to the dose-dependent rise in plasminogen activator production (unpublished results of R. D. Ginzberg).

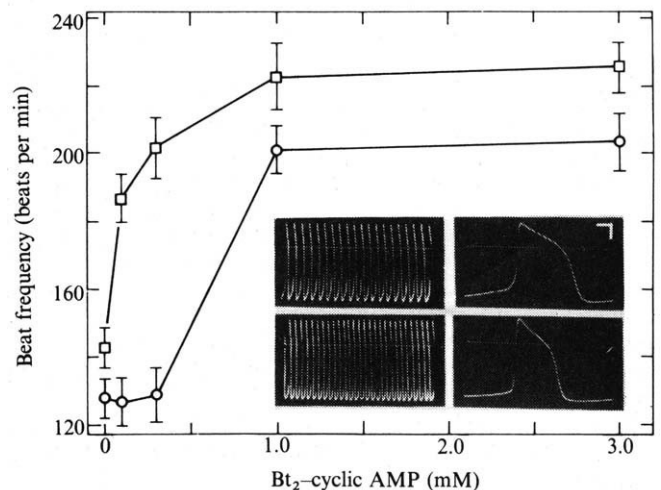
Communication in cocultures

Cocultures of granulosa cells and myocardial cells were obtained by adding a suspension of freshly prepared granulosa cells to myocardial cells that had been in culture for 24–72 h. Communication between heterologous cells in the cocultures was characterised by electron microscopy, autoradiography and electrophysiology (Fig. 3).

Granulosa cells could be identified readily in the cocultures on the basis of several cytological properties. One of the most distinctive ultrastructural features of granulosa cells both *in vivo* and in culture is the annular gap junction^{32–34}. Other distinguishing characteristics include cytoplasmic granules and a large ratio of nucleus to cytoplasm volume. On the other hand, myocardial cells in culture possess characteristic thick and thin myofilaments that are partially organised into sarcomeres. In addition, myocardial cells are larger than granulosa cells and contain a higher density of mitochondria. Using these criteria for cell identification, gap junctions have been detected routinely between granulosa and myocardial cells in coculture (Fig. 3a).

Here, as in several other cell culture systems, gap junctions are associated with the ability to transfer labelled nucleotides between

Fig. 2 Effect of Bt₂-cyclic AMP on the beat frequency and action potential of cultured myocardial cells. Conditions were the same as in Fig. 1. Each value is the mean $\pm$ s.e. of six cultures. □, Cultures were preincubated for 20 min in $2 \times 10^{-5} \text{ M}$ MIX, while the other cultures (○) were incubated without MIX. The two upper oscilloscope traces were recorded before addition of 1 mM Bt₂-cyclic AMP; the two lower traces were obtained approximately 10 min after addition. The calibration marks and bath potential are as indicated in Fig. 1, except the horizontal bar is 2 s for the left traces and 100 ms for the right traces.



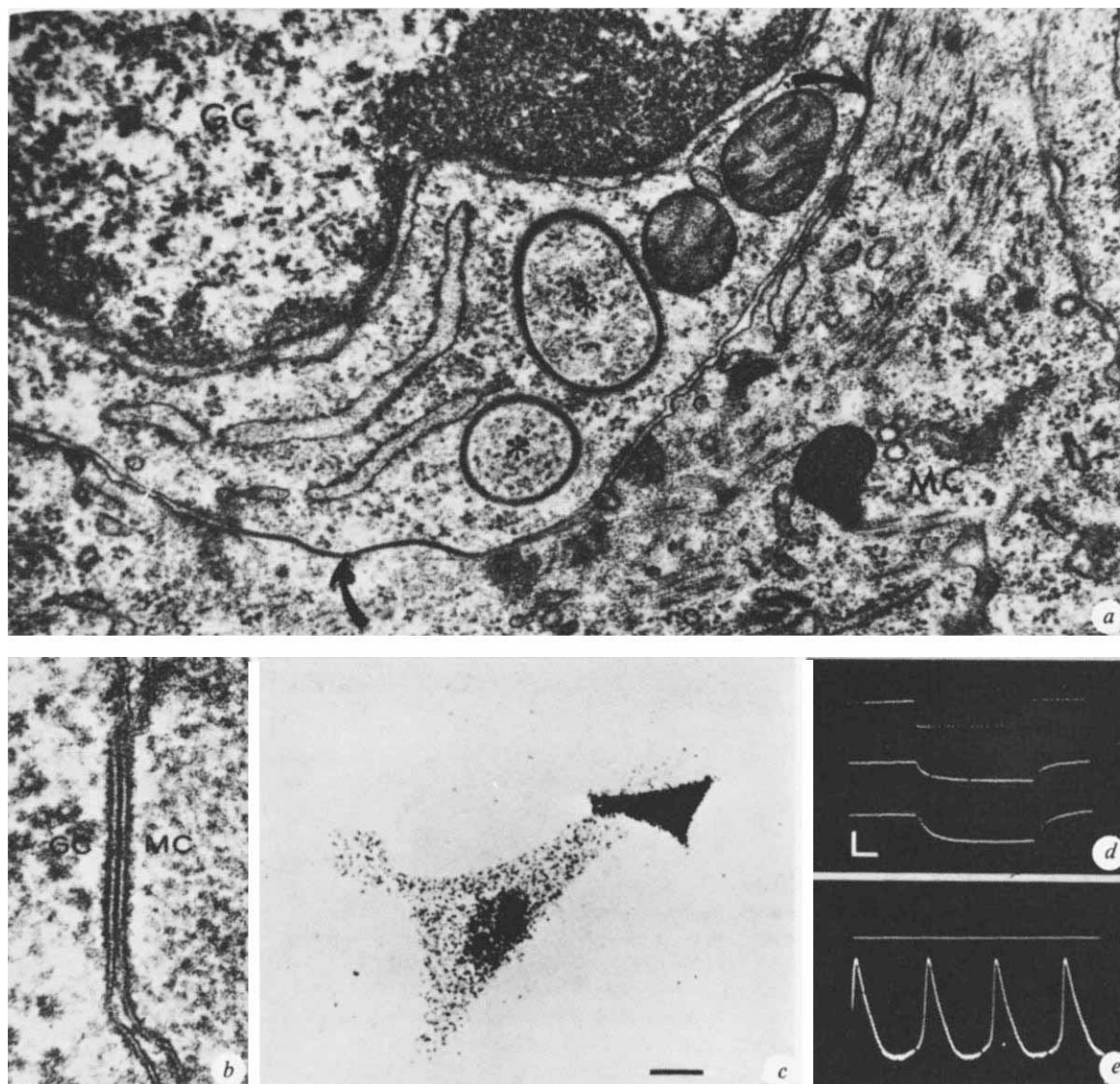


Fig. 3 Communication between heterologous cells in coculture. *a*, Demonstration of gap junctions between granulosa cells and myocardial cells. Cocultures were prepared for electron microscopy as described by Kalderon *et al.*⁴⁰. The heterologous cells are joined by extensive gap junctions (large arrows). Two annular gap junctions (asterisks) are present in the cytoplasm of the granulosa cell (GC), and the characteristic myofibrils (MF) of the adjacent myocardial cells (MC) are evident. Asymmetrical desmosomes (D) are also generated between heterologous cells; there is greater submembranous density in the myocardial cell ($\times 25,000$). *b*, High magnification image of a gap junction between heterologous cells. There is no apparent asymmetry in the junction between granulosa (GC) and myocardial (MC) cells ($\times 160,000$). *c*, Metabolic coupling between granulosa and myocardial cells. Fresh suspensions of granulosa cells were incubated for 3 h at 37 °C in L-15 supplemented with 10% calf serum and ^3H -adenine ($10 \mu\text{Ci ml}^{-1}$ specific activity, 15Ci mmol^{-1}). The cells were washed four times and added to cultures of myocardial cells cultured for 48–72 h were incubated with ^3H -adenine or ^3H -uridine. When granulosa cells were added to the culture, label was transferred readily to contacting cells. The bar represents 10 μm . *d* and *e*, Ionic coupling between granulosa and myocardial cells. *d*, Cocultures were monitored for ionic coupling. The top trace indicates the current pulse. The middle trace records the voltage in the impaled granulosa cell. The bottom trace measures the voltage in the myocardial cell. Current injected into the myocardial cell through an active bridge circuit caused a voltage deflection in the adjacent granulosa cell. *e*, Rhythmic depolarisations in a granulosa cell that was ionically coupled to beating myocardial cells. Vertical calibration bar is 10 mV for both records and 0.5 nA for record (*d*). Horizontal bar is 50 ms for record (*d*) and 1 s for record (*e*).

contacting cells (metabolic coupling)¹⁰. This was demonstrated by prelabelling either granulosa cells or myocardial cells with ^3H -adenine or ^3H -uridine. After extensive washing to remove unincorporated extracellular label, the prelabelled population was cocultured with unlabelled heterologous cells. Label was transferred to the heterologous cells only if they were in contact with prelabelled cells (Fig. 3c). Each cell type could act as a competent donor, indicating that transfer of molecules of this size is bidirectional.

The presence of ionic coupling was detected readily between heterologous cells, consistent with the ultrastructural observations and the nucleotide transfer data. Injection of current into a myocardial cell caused a voltage deflection in a neighbouring granulosa cell (Fig. 3d), demonstrating that a low resistance pathway was present between these cells. Furthermore, granulosa cells in coculture frequently synchronised myocardial cells that

were not in direct contact with each other. Finally, the synchronising granulosa cells depolarised with each myocardial cell action potential (Fig. 3e). These observations on granulosa cells cocultured with myocardial cells are similar to those of Hyde *et al.*³⁵ on heart-derived fibroblasts and of Goshima³⁶ on FL cells.

Although these cells influence one another in coculture, it is important to determine whether this is due to contact-dependent cell communication where each cell maintains its identity, or to cell fusion, where the membranes and cytoplasmic contents of the heterologous cells are mixed. Two lines of evidence suggest that fusion does not occur in these cocultures. (1) The frequency of binucleated cells (an indication of fusion) was not greater than that of myocardial cells cultured alone (less than 0.1%). In addition, multinucleated cells have never been observed. (2) When granulosa cells which had been loaded with latex beads and labelled with ^3H -uridine were cocultured with myocardial cells,

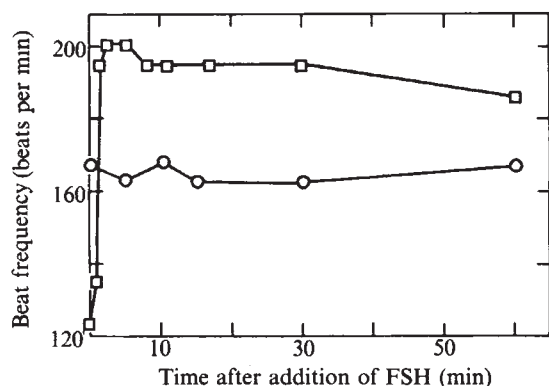


Fig. 4 Effect of FSH on myocardial cells and cocultures. Myocardial cells (5×10^4) were plated into adjacent wells of a Falcon multiwell plate and 24–72 h later, 3×10^5 granulosa cells were added to one of the two wells. The medium was removed 4 h later, then the wall between the wells was removed and L-15 supplemented with 10% calf serum was added. FSH ($10 \mu\text{g ml}^{-1}$) was added to the cultures 14–18 h later. The myocardial cells cultured separately (○) were unaffected while there was a rapid increase in the beat frequency of the myocardial cells cocultured in the adjacent well (□). In another experiment, cyclic nucleotide phosphodiesterase (Sigma) sufficient to hydrolyse 10 nmol of cyclic AMP per min was added to cocultures before the FSH. Because rat granulosa cells produce approximately 5–10 pmol of cyclic AMP per 10^6 cells during the first hour after exposure to FSH ($10 \mu\text{g ml}^{-1}$) (unpublished results of R. D. Ginzberg) this amount of enzyme should have been sufficient to hydrolyse any secreted cyclic AMP. This treatment did not affect the increase in beat frequency resulting from addition of FSH to cocultures. The FSH preparation used was NIH-FSH S-11. The LH mentioned in the text was NIH-LH S-19 and the PMSG was a highly purified preparation provided by Drs William T. Moore and Darrell Ward of the University of Texas.

the nucleotide was transferred readily between the heterologous cells without the transfer of latex beads. For these experiments, granulosa cells were cultured in plastic tubes for 12–24 h with 2.0- μm diameter latex beads (Dow Corning). After the granulosa cells had incorporated beads into their cytoplasm, they were labelled with ^3H -uridine (for methods see legend to Fig. 3c) and cocultured for 12–18 h with myocardial cells. The latex beads were clearly segregated in the granulosa cells in all of the 500 examined cases of nucleotide transfer between the heterologous cells.

Stimulation of cocultures by FSH

As demonstrated above, myocardial cells in culture respond to noradrenaline with an increase in beat frequency and action potential amplitude and a decrease in action potential duration. These parameters were monitored during addition of FSH to cocultures to determine whether this treatment led to changes similar to those observed when myocardial cells were exposed to noradrenaline. The addition of FSH to cocultures led to a rapid increase in the myocardial cell beat frequency (Fig. 4), and the increase in beat frequency was dependent on the concentration of FSH (Fig. 5). The dose dependence of this response resembled that of the FSH-induced increase in synthesis of plasminogen activator by granulosa cells (Fig. 5), although production of plasminogen activator was more sensitive to FSH by a factor of 3–5. Concomitant with this increase was an increase in action potential amplitude and a decrease in action potential duration. (Treatment with FSH ($10 \mu\text{g ml}^{-1}$) caused an action potential duration of $88 \pm 2\%$ ($n = 14$) and an amplitude of $105 \pm 1\%$ ($n = 14$)). Luteinising hormone (LH) and pregnant mare serum gonadotropin (PMSG) also caused an increase in beat frequency in coculture. PMSG was more potent than FSH, which was in turn more potent than LH. This same order of potency was observed when these preparations were used to induce production of plasminogen activator in granulosa cells.

The ratio of cell types in the coculture strongly influenced the response. Cocultures with ten or more granulosa cells per myocardial cell (10:1) exhibited no spontaneous beating and appeared unaffected by exposure to FSH. This observation is consistent

with the report³⁵ that myocardial cells cocultured with a large excess of fibroblasts beat sluggishly if at all. FSH-induced increases in beat frequency were observed only when the ratio of granulosa to myocardial cells was in the range of 2:1 to 6:1.

The FSH-induced increase in beat frequency is dependent on contact between the heterologous cells (Fig. 4). Granulosa cells were seeded into a culture of myocardial cells. A physical barrier was constructed in the culture dish so that the coculture was able to share medium with, but not contact, a separate myocardial cell culture. Addition of FSH caused a rapid increase in the beat frequency of only those myocardial cells that were in direct contact with granulosa cells. There was no change in the beat frequency of the myocardial cells that had only shared medium. In a related experiment, external cyclic nucleotide phosphodiesterase had no effect on the FSH-induced increase in beat frequency (Fig. 4). This suggests that secreted cyclic AMP is not responsible for the observed response.

Although our observations demonstrate that cell contact is required for the increase in beat frequency, they could be explained by hypothesising an FSH-induced change in the electri-

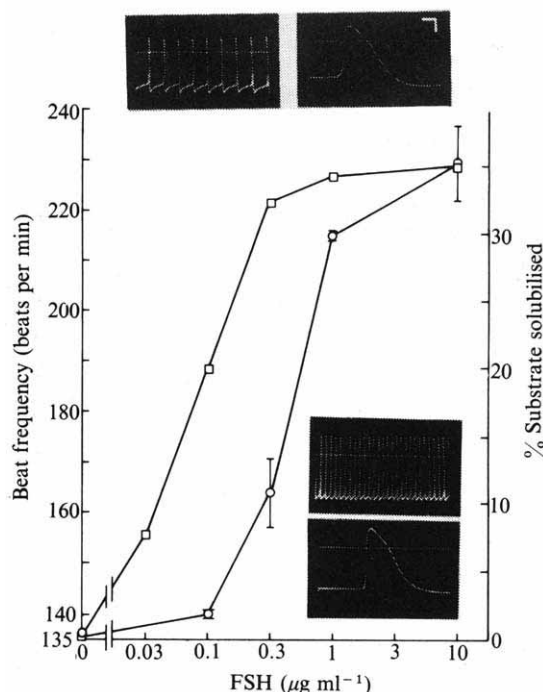


Fig. 5 Comparison of FSH-induced synthesis of plasminogen activator in granulosa cells and of stimulation of beat frequency in cocultures. Granulosa cells prepared according to Beers *et al.*³⁰ were plated on 35-mm Falcon dishes at a density of 10^6 cells per dish in L-15 supplemented with 10% plasminogen-depleted foetal bovine serum and incubated at 37°C in air. After 3 h the cells were washed, the indicated concentrations of FSH were added, and the cultures were incubated for a further 4 h. The cultures were then washed twice in 0.1 M Tris-HCl (pH 8.1), and lysed with 0.1% Triton in 0.1 M Tris. The lysates were assayed as described by Unkless *et al.*³⁹. □, Intracellular level of activator. The results are presented as the fraction of available substrate. For beat frequency studies, conditions were similar to those described in Fig. 4. Beat frequency was monitored during the first 10 min after addition of FSH and the peak frequency attained during this period is plotted as a function of FSH concentration. Values reported (○) are the mean $\pm$ s.e. ($n = 4$). For electrophysiological studies, 1×10^5 myocardial cells were plated into a chamber as described in Fig. 1. The ring was replaced just before addition of 2×10^5 granulosa cells, and removed after 4–6 h, when further L-15 was added to the cultures. Cultures were used 12–18 h later for electrophysiology. All data were derived from continuous impalement of one cell before and after FSH addition to the bath. The upper oscilloscope traces were recorded from a myocardial cell before treatment of cocultures with FSH ($10 \mu\text{g ml}^{-1}$). Those in the lower right were obtained approximately 10 min after addition of FSH. The calibration bars are the same as in Fig. 1 except that the horizontal bar represents 2 s in the left trace of the top pair and the upper trace of the bottom pair, while it represents 50 ms for the other traces.

cal properties of granulosa cells. Because the stimulation is observed between cocultured granulosa and myocardial cells that are ionically coupled, a change in the membrane resistance of granulosa cells could conceivably affect the frequency of myocardial cell beats. Indeed, unstimulated cocultures beat more slowly than myocardial cells cultured alone (Fig. 4), perhaps because the granulosa cells act to shunt the myocardial cell pacemaker current. If FSH increased granulosa cell membrane resistance, this might act to decrease the shunt and increase beat frequency. To test this possibility, the membrane resistance and potential of granulosa cells cultured alone were studied during hormonal stimulation. A single microelectrode with an active bridge circuit was used to monitor continuously cells both before and after addition of FSH. Small current pulses (< 0.5 nA) were used to maintain proper bridge balance. No change in either membrane resistance or potential occurred during the first 10 min after addition of FSH. In these conditions changes exceeding approximately 20% of the initial resistance would have been detected. Experiments with separate current passing and voltage recording electrodes are in progress to confirm these observations.

The beat frequency observed when cocultures were exposed to saturating levels of FSH exceeded the frequency of the same number of myocardial cells cultured alone (Fig. 4). Therefore the increase in beat frequency cannot be interpreted simply as the removal of the inhibition of the spontaneous beat frequency produced by granulosa cells. In fact the increase in beat frequency can approach that induced by treatment of cocultures with saturating levels of noradrenaline and Bt_2 cyclic AMP. In one experiment cocultures were stimulated with either FSH ($10 \mu\text{g ml}^{-1}$), 2×10^{-6} M noradrenaline or 5 mM Bt_2 cyclic AMP. After these additions, frequencies increased from 107 ± 2 ($n = 12$) to 171 ± 3 , 183 ± 2 , and 179 ± 9 beats per min, respectively ($n = 4$ for each).

Granulosa cells cocultured with myocardial cells retained the ability to respond to FSH by producing plasminogen activator. Although the dose dependence of this response was the same as when granulosa cells were cultured alone, in coculture there was a reduction in the level of enzyme synthesised and the amount secreted into the culture medium. Exposure of the cells to a cyclic nucleotide phosphodiesterase inhibitor such as MIX resulted in an increase in the synthesis of activator to near control levels. However, in these conditions secretion was still inhibited.

Stimulation of cocultures by noradrenaline

Plasminogen activator was assayed in cocultures that had been exposed to noradrenaline and MIX to determine whether myocardial cells could mediate the stimulation of granulosa cells. These experiments demonstrated that the cocultures synthesise plasminogen activator when exposed to concentrations of noradrenaline and MIX that caused myocardial cells to beat more rapidly (Fig. 6). The addition of MIX was required in order to observe this effect. The dose dependence of this response was the same as the noradrenaline-induced increase in beat frequency.

Experiments similar to those described above demonstrated that this result was also dependent on cell contact. A coculture of myocardial and granulosa cells was established on one side of a physical barrier and granulosa cells were cultured on the other side. The barrier allowed the cultures to share medium, but did not permit cell contact. Addition of noradrenaline and MIX to the medium common to all the cells resulted in increased levels of plasminogen activator only in cocultured cells. Cyclic nucleotide phosphodiesterase also had no effect on noradrenaline-induced synthesis of plasminogen activator in cocultures.

It should be noted that in certain conditions, granulosa cells cultured in medium containing MIX respond to high levels of noradrenaline ($\geq 10^{-6}$ M) with measurable activator synthesis²⁹. Activator synthesis in cocultures, however, was routinely detected at much lower doses (10^{-9} M noradrenaline) which permitted a clear distinction between direct stimulation of the granulosa cells and the stimulation of activator synthesis that was mediated by the myocardial cells.

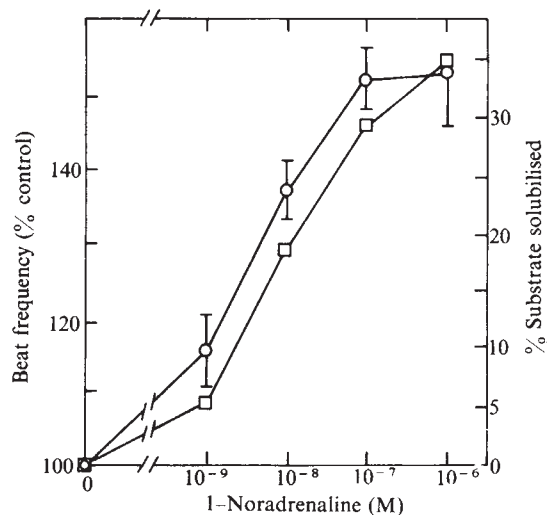


Fig. 6 Comparison of noradrenaline-induced stimulation of beat frequency in myocardial cells and synthesis of plasminogen activator in cocultures. Myocardial cells were plated at 10^6 per 35-mm dish. Cocultures were established by addition of 10^6 granulosa cells per dish, 48 h after myocardial cell plating. After 7 h in coculture, various concentrations of noradrenaline were added along with 3×10^{-5} M MIX. The cultures were lysed 4 h later and plasminogen activator was assayed as in Fig. 5 (□). Myocardial cells cultured alone contained no detectable plasminogen activator after exposure to noradrenaline and MIX. Beat frequency was determined with myocardial cells cultured alone as in Fig. 1 except that cultures were pre-incubated for 20 min in 2×10^{-5} M MIX (O). All values are the mean $\pm$ s.e. ($n = 3$).

Myocardial cells in coculture responded to noradrenaline by increasing their beat frequency with the same dose response characteristics as myocardial cells cultured alone; however, the spontaneous beat frequency of the cells in coculture was slower. As mentioned above, it is possible that this is the result of a granulosa cell shunt of the myocardial cell pacemaker current.

Mechanism of communication

We have demonstrated that hormonal stimulation can be communicated between granulosa and myocardial cells by a contact-dependent mechanism. These two cell types form gap junctions in coculture and are metabolically and ionically coupled. The presence of intercellular communication between the heterologous cells provides a plausible mechanism to account for the response of cocultured cells to hormonal stimulation: namely that a molecule (or molecules) is produced on exposure of granulosa cells to FSH and of myocardial cells to noradrenaline, and that this can move from one cell type to the other through gap junctions. The transmitted molecule then causes a response in the heterologous cell type which, if cultured alone, is unresponsive to the hormone. The proposed communicator molecule must be small and capable of initiating plasminogen activator synthesis in granulosa cells, and of altering the action potential and beat frequency of myocardial cells. A likely candidate for this substance is cyclic AMP but more evidence is required before accepting this as the communicator of hormonal stimulation.

Several other explanations seem less likely to account for the observed effects. The experiments of Schramm *et al.*³⁷, suggest that if massive fusion were taking place in cocultures of granulosa and myocardial cells, results similar to those presented here might be observed. However, because fusion has never been detected, we feel that this is an unlikely explanation. It might be possible that even in the absence of fusion, receptors migrate from one cell type to the other in the regions of cell contact. We know of no experimental precedent for the migration of an integral membrane protein from cell to cell by cell contact. However, experiments using ^{125}I -gonadotropins are in progress which may enable us to detect such transfer if it occurs.

Thus, based on the data presented here, the most probable mechanism to account for the cross-stimulation observed in

cocultures is gap junctional transfer of a communicator of hormonal stimulation. If this hypothesis is supported by further investigation, it is possible that the phenomenon of intercellular communication may have a biologically relevant role in the transmission and regulation of hormonal stimulation in various mammalian organs.

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Three-dimensional reconstruction of the fibres of sickle cell haemoglobin

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Three-dimensional reconstruction of electron micrographs of the 20-nm diameter fibres of HbS reveals an inner helical core of four strands surrounded by an outer helix of 10 strands to give a total of 14 strands. The strands are arranged with roughly hexagonal packing to produce an unusual helical structure which features a variety of intermolecular contacts and a non-circular cross section.

SICKLE cell disease has long been recognised¹ and the role of a haemoglobin variant in the disease was specified in 1949 (ref. 2). An active effort to understand the structure of the fibres of haemoglobin S that lead to the symptoms of the disease has occurred only recently. Structural studies^{3–9} with X rays and electrons have led to the proposed specific structural models. Two distinct forms of fibres of haemoglobin S have been identified by electron microscopy on negatively stained samples—17-nm diameter fibres with striations perpendicular to the fibre axis⁴ and 20-nm diameter fibres with striations diagonal to the fibre axis^{5,7–9}. In studies with sickled cells, both classes of fibres were observed, but the 20-nm diameter fibres were found in much greater abundance⁷. In studies on fibres from gelled haemolysates, only the 20-nm diameter fibres have been observed^{5,8,9}. It therefore seems that under most conditions the 20-nm diameter fibres are the predominant form. In this report we describe the analysis of these fibres with three-dimensional image reconstruction methods, using Fourier transform techniques^{10–12}. For the first time an inner core structure for the fibres is revealed.

Analysis of the 20-nm diameter fibres of haemoglobin S has gone through several stages as the quality of micrographs improved and the analysis progressed from real space measurements⁵ to optical transforms^{7–9}, and now to computer reconstructions. The initial optical transforms revealed meridional reflections at about 1/30 Å, pairs of reflections at about 1/60 Å, and pairs of reflections close to the equator. A splitting of the 1/30 Å reflection was observed with fibres prepared from haemolysates^{8,9}, and the splitting has also been apparent in fibres from sickled cells. Various forms of polymorphism within the 20-nm diameter fibres have been considered, but we are now inclined to the view that the 20-nm diameter fibres are a homogeneous class, but of such complexity that very long regions of fibres must be examined. This view is strengthened by our work with computed transforms in which a consistent pattern of reciprocal maxima can be obtained from fibres prepared either from sickled cells or gelled haemolysates. The Fourier transforms give a highly complex pattern of reciprocal space maxima with approximately 30 resolvable maxima on 20 layer lines, suggesting that interpretations from the less well resolved optical transforms were incomplete and variable. The Bessel orders to each of the maxima of the transform have now been assigned, permitting three-dimensional reconstructions of the fibres to be carried out. The results of these reconstructions are presented here.

Images of the fibres and their Fourier transforms

Electron microscopy images of the fibres of haemoglobin S were recorded on samples negatively stained with 2%

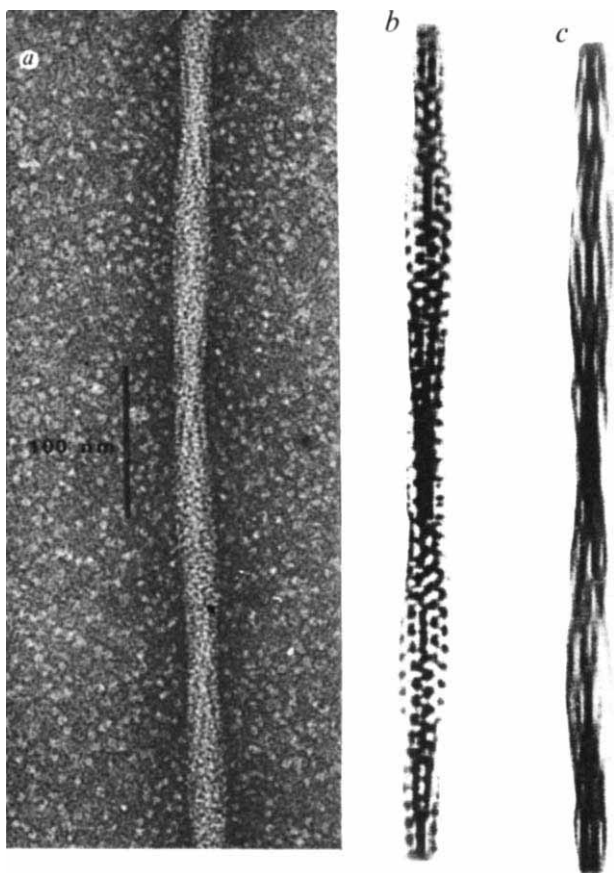


Fig. 1 Negatively stained image and reconstructions of fibre of HbS. *a*, Micrograph of fibre of HbS prepared from a sickled cell by direct lysis with negative stain on the electron microscope grid. *b*, Two-dimensional reconstruction of the fibre of HbS using computer reconstruction techniques with the maxima from the Fourier transform displayed in Fig. 2. The output is recorded from a Tektronix graphics terminal. *c*, Two-dimensional reconstruction as in (*b*), but with only the maxima of layer lines 1–6 of the Fourier transform used.

phosphotungstate on high resolution grids, as described previously⁹. A Philips EM 301 electron microscope was used and images were recorded with minimum beam exposures. A typical fibre is presented in Fig. 1 and shows the periodic variation in apparent diameter that is a consistent feature. Short regions of striations parallel to the fibre alternating with regions having a more cross-hatched appearance are also evident. To compute Fourier transforms of the fibres, electron microscope plates were digitised with a Syntex AD-1 autodensitometer. This instrument was interfaced to a Nova computer equipped with tape and disk drives and a Tektronix graphics terminal, permitting the digitisation, transformation and reconstruction to be carried out on the same computer. The development of this on-line image reconstruction system has greatly facilitated our studies, enabling us to easily compute numerous transforms on many different fibres. Only after a consistent Fourier transform pattern of amplitudes was found repeatedly were we prepared to accept its validity, since it has several uncommon features. A transform showing these various features is reproduced in Fig. 2. Many maxima are present clustered into three regions: a region near the equator, a region in the middle of the transform (around layer line 24) corresponding to spacings in the vicinity of $1/60$ Å, and a region near the top of the transform (around layer line 49) corresponding to spacings in the vicinity of $1/30$ Å. These same regions were identified in earlier optical transforms^{7–9}, but the complexity of the various maxima within each region was not appreciated, since it was beyond the resolv-

ing power of the optical diffractometers used. Two-dimensional reconstructions, which do not require assignment of Bessel orders, give good agreement with original fibres, as seen in Fig. 1*b*. Thus, the Fourier amplitude map shown in Fig. 2 represents the 20-nm diameter fibres and the next step was to evaluate the information contained in the map.

Assignment of Bessel orders

For most of the fibre structures of proteins previously analysed by Fourier methods, patterns considerably less complex than Fig. 2 were obtained¹² and the maxima could be reconciled with a single helical lattice. In the fibres of haemoglobin S the reciprocal space pattern is composed of contributions from several overlapping families of lattices, including contributions from structural elements at the interior of the fibres. The final assignment of Bessel orders to each maxima was therefore a difficult process and several approaches were used (a detailed description of each approach will be published elsewhere).

First, the question of whether a particular maximum arose from an even-ordered or an odd-ordered Bessel function was determined in the usual way¹¹: by evaluating whether the left and right members of each pair were in phase (corresponding to even order) or out of phase (corresponding to odd order). The results of this analysis were unambiguous and indicated that the maxima in the central region of the transform (layer lines 21–28) were all of odd order, whereas all other maxima (on layer lines 1–6 and 46–51) were of even order.

Second, efforts were directed at assigning the absolute Bessel order for a given maximum as the ratio of phase change per degree of tilt (parallel to the fibre axis) for micrographs recorded at various angles with the goniometer stage, as described by Finch¹³. This method was only partially successful, principally because it required recording several images from each fibre and the quality of the computed transforms decreased markedly with successive exposures. Nevertheless some useful information was obtained, particularly for the maxima near the equator, for which the data indicated a trend from low to high Bessel orders with increasing layer line number (for layer lines 1–6). In addition, the same Bessel order was indicated for the multiple maxima of a given layer line.

Third, we developed a new approach to assigning Bessel orders based on a combination of real space and Fourier space reconstruction methods. The basis of this approach was to compute a two-dimensional reconstruction of the fibres using only the reflections from layer lines near the equator (1–6) to give a reconstructed analogue of the fibre with an array of continuous strands, as shown in Fig. 1*c*. This idealised fibre had an apparent repeat of 3,000 Å. A real space algorithm^{14,15} was then used to deduce the cross section of the idealised fibre, assuming that each increment along the length of the fibre corresponded to a specified degree of rotation. The outcome was an elliptical cross section composed of four inner strands and 10 outer strands. Transforms of structural models based on this pattern gave near-equatorial reflections similar to those seen on the transforms of haemoglobin S fibres and rotations of the model permitted the Bessel orders of the reflections to be assigned¹³. The rotations indicated that layer lines 1–6 contained reflections of Bessel orders 2, 4, 6, 8, 10 and 12, respectively. Once these assignments were made, the Bessel orders of the reflections on the higher layer lines could also be determined, using the traditional reasoning of lattice building. For example, layer lines 5, 27 and 49 can be connected by a lattice line with maxima of Bessel orders 10, 5 and 0 on the three layer lines, respectively. The various assignments are summarised in Table 1. In effect, a distinct reciprocal space lattice can be drawn for each of the 12 distinct maxima on each side of the first six layer lines

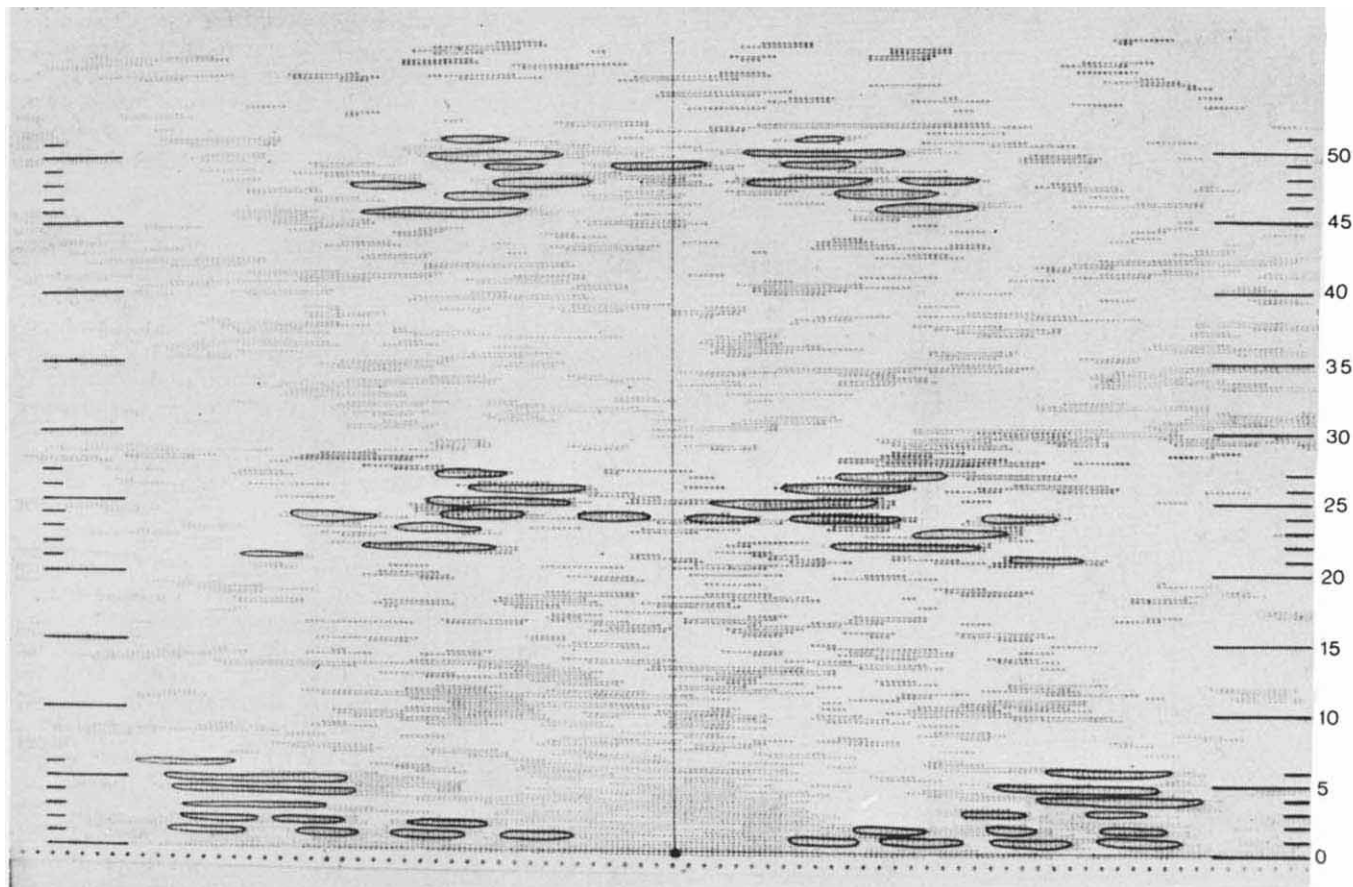


Fig. 2 Fourier transform amplitude map of fibre of HbS. The map was obtained by computer transform of a 256×256 array of densities corresponding to a region of the HbS fibre digitised on the Syntex AD-1 autodensitometer. The computations were performed on a Nova computer and the output displayed as integers corresponding to increasing density levels on a high-speed printer. Regions of density above background were outlined in black to aid visualisation and the various layer lines of the maxima are numbered on the right. The first layer line corresponds to a spacing along the fibre axis of 1,500 Å.

with other points of the reciprocal space lattices lying among the maxima on layer lines 21–28, and with additional lattice points on layer lines 46–51. The clusters at the middle and higher ranges of lattice lines contain fewer maxima than in the near-equatorial region, since lattice points from more than one lattice are superimposed in several cases among the maxima in the middle and upper regions of the transform.

Three-dimensional reconstructions

The arrangements of strands can be visualised by reconstructing the near-equatorial reflections in three-dimensions. Such a reconstruction is shown in Fig. 3. The cross-section indicates that the fibres are packed with individual strands in a hexagonal array. An important feature of the structure is that the four inner strands are not equivalent, but occur in two pairs. Thus, the distance between two of the inner strands (11 and 13 in Fig. 3) is greater than the distance between the other two inner strands (12 and 14 in Fig. 3). The outer strands also occur with a family of radial spacings. This inequivalence in the radii of the strands results in a noncircular, roughly elliptical cross section.

When three-dimensional reconstructions are computed using the reflections of Table 1 to 60 Å resolution, density varies along the length of each of the 14 strands and the appearance of the sections depends on the position along the fibre axis. By following individual strands through many such sections and noting the level of maximum density, the locations of molecules along the strands of the inner and outer core can be deduced. This information is summarised in the form of a surface lattice in Fig. 4 and as a

sphere model in Fig. 5. Data at higher resolution (to 30 Å) may reveal subunit orientations in addition to the location of molecules and this point is currently under investigation.

Fig. 3 Three-dimensional reconstruction of fibre of HbS. This reconstruction was computed using the maxima of layer lines 1–6 (corresponding to the two-dimensional reconstruction of Fig. 1c) in order to permit each of the 14 strands to be clearly displayed in the same reconstruction. In reconstructions which also include maxima from higher layer lines, densities from certain strands are reduced or absent and compilations of many such reconstructions from different positions along the fibre axis permitted the overall helical pattern of the fibre to be specified, as displayed in Fig. 4. This figure is a photograph from the Tektronix terminal with densities displayed in terms of contour lines.

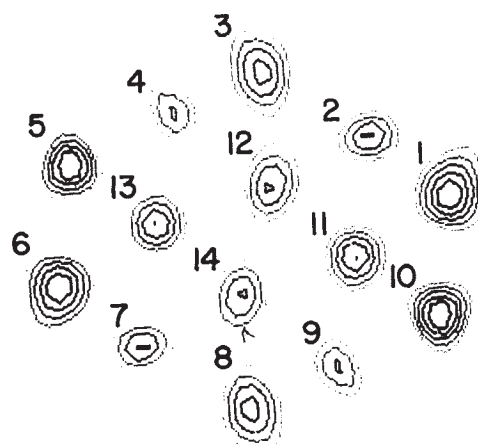


Table 1 Layer lines and Bessel orders for Fourier transforms

Layer line	Bessel order	No. of maxima
1	2	4
2	4	3
3	6	2
4	8	2
5	10	1
6	12	1
21	-7	1
22	-5	1
23	-3	2
24	-1	3
25	1	1
26	3	1
27	5	1
28	7	1
46	-6	1
47	-4	2
48	-2	2
49	0	2
50	2	1
51	4	1

The Bessel orders are as assigned to the maxima of the respective layer lines of the transforms of the fibres. For layer lines with more than one maxima, as indicated in the column on the right, each maxima was characterised by the same Bessel order. These data are a composite from several transforms, as individual transforms may have one or more reflections absent.

Implications of the 14-stranded structure

The long-range goal of the structural studies on fibres of haemoglobin S is to bring the analysis to atomic resolution, so that the particular amino acid residues at each intermolecular contact can be specified. The complexities in the structure of the fibres revealed in this report suggest that description of the intermolecular contacts in atomic terms will be more difficult than previously imagined. It is thus not surprising that the list of sites on the haemoglobin S molecule where changes can alter fibre formation is growing¹⁶⁻²¹. It now seems likely that many positions on

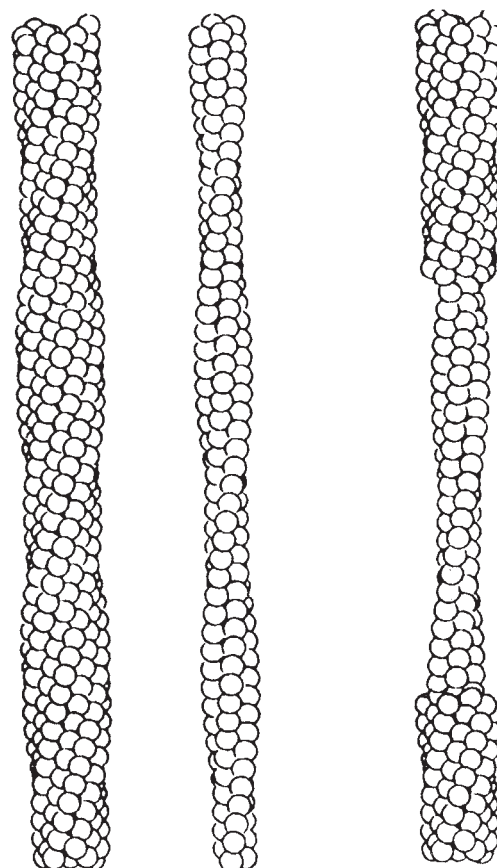
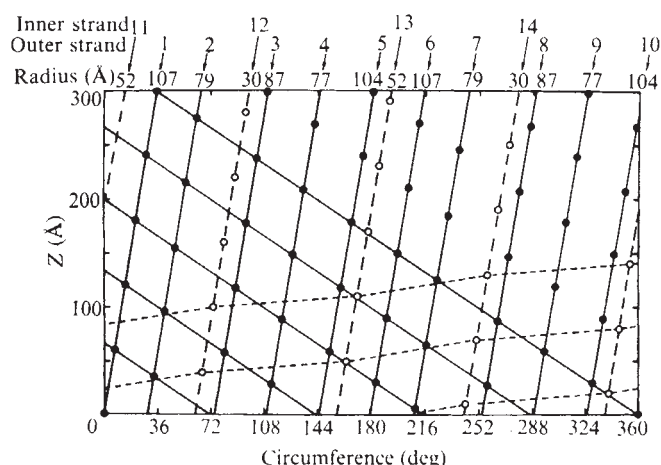


Fig. 5 Solid sphere models of the helical elements of the fibres of HbS. The models present the outer strands (on the left); the inner strands (in the centre) in alignment with the outer strands such that the narrow diameter region of the inner strands corresponds to the narrow diameter region of the outer strands, and a cutaway version (on the right) showing both inner and outer strands in proper juxtaposition.

Fig. 4 Surface lattice of the fibres of HbS. The 14 strands of the fibres are displayed with a numbering system corresponding to the designations in Fig. 3. For each strand the centre of its radial distance is indicated at the top of the surface lattice. The outer 10 strands are represented with filled circles and the inner 4 strands with open circles. Because of the differences in the radii of the strands, the lines of the surface lattice cannot all be straight. The data are not sufficiently precise to specify exactly the small perturbations from linearity, so the surface lattice is arbitrarily drawn with the left-handed 5-start diagonal lines linear. The inner four strands occur at two pairs of radii such that a pseudo 1-start helix (dashed line) has a slope which alternates between two angles, which differ slightly, at each intersection with an inner strand.



the molecule participate in the self-association process involved in fibre formation once the thermodynamics have been tipped towards fibre formation by the primary Glu→Val transition. A large number of distinct classes of intermolecular contacts are implied by the variety of nearest-neighbour interactions, but some simplifications in the distinctions between contacts may develop through application of the principle of quasi-equivalence which has been useful in other supramolecular structures, particularly viruses.

The new structure of the fibres may also help to explain the exceptionally high order in the kinetics of fibre assembly²²⁻²⁴ and the high density of gels²⁵. Our preliminary calculations based on the 14-stranded model indicate that the densities of closely packed fibres are consistent with the fibre pellet concentrations reported by Ross *et al.*²³. In fact, the close hexagonal packing of the individual strands is an arrangement which may maximise the quantity of protein in a volume element of the fibres. It remains to be seen whether similar packing will emerge as a general pattern for helical protein assemblies. One example already reported is the seven-stranded cable of glutamine synthetase²⁶ which has a hexagonal cross-section. The advantages of hexagonally packed cables have been appreciated for some time²⁷⁻²⁸, particularly units with three strands and seven strands²⁶. If this series is extended, 10-stranded and 14-stranded structures would also be predicted, with the HbS structure presented here as an example of the latter.

The complexities of the 14-stranded structure now make it less likely that data on intermolecular contacts from crystals of single molecules of haemoglobin S⁶ will be directly applicable to the intermolecular contacts of the

fibres. However, the fact that the bundles formed by stirring haemolysates²⁹ are crystalline arrays of fibres in the form described here⁹ offers a possibility of obtaining data to higher resolution by crystallographic means which, in conjunction with existing atomic models for individual molecules of HbS⁶, could be sufficient to specify molecular orientations within the fibres and the stereochemistry of the contacts.

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Gene *K*, a new overlapping gene in bacteriophage G4

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A third overlapping gene in the isometric bacteriophages has been identified by nucleotide sequence analysis of the G4 genome and by isolation and microsequence analysis of the new protein. Five nucleotides are used in all three translational reading frames of the DNA.

THE single-stranded DNA bacteriophage G4 is closely related to phage ΦX174 (ref. 1); its genome has the same overall structure as that of ΦX174 (J.C.F., B.G.B. and G.N.G., unpublished), and despite a 40% base sequence difference, codes for a similar set of 10 proteins.

The complete nucleotide sequence of ΦX174 revealed that 2 of the 10 genes so far identified overlap and are translated in a different reading frame²⁻⁴. An examination of all three translational reading frames of the complete sequence has revealed several other possible overlapping genes (coding for proteins of more than 20 amino acid residues) which have the following characteristic features⁵. An open translational reading frame of at least 60 nucleotides comes before a termination codon in the same phase, and the ATG initiation codon is preceded by a sequence complementary to the 3' end of the 16S ribosomal RNA. This presumed ribosome-binding site should be at least three nucleotides long and be within 15 nucleotides of the initiation codon^{6,7}. Sequences very similar to at least two of these putative genes have also been found in phage G4 (J.C.F., B.G.B. and G.N.G., unpublished).

We now provide evidence from nucleotide sequencing combined with protein chemistry, for a hitherto unknown phage-specified protein whose amino acid sequence corresponds to one of these nucleotide sequences.

This gene has been named *K* following the convention adopted for ΦX174. Gene *K* overlaps genes *B*, *A* and *C* and is the third example of an overlapping gene in the isometric phages. Two regions of gene *K* briefly overlap with two other genes simultaneously so that all three translational reading frames of the DNA can be used.

DNA sequence analysis of gene *K*

Inspection of the DNA sequence of bacteriophage ΦX174 (ref. 5) in the gene *A/B* and *C* region revealed a potential new translational reading frame extending from the ATG at nucleotide 51 to the TGA termination codon at position 210. The ATG was preceded by a GGA sequence eight nucleotides upstream which is complementary to the nucleotide sequence shown boxed at the 3' end of 16S rRNA, that is, 5' GAUACAC-UCCUUA_{OH}3'. This sequence has been shown to be involved in the initiation of protein synthesis^{8,7}. This possible new gene (gene *K*) would code for a protein of 56 amino acids. The initiation codon overlaps the termination codon of gene *B* and extends through the end of gene *A* into gene *C*.

To see if this potential gene is conserved in the related bacteriophage G4 the DNA sequence in this region was determined using the 'plus and minus' method, and more recently, the new chain termination method of Sanger *et al.*⁸. Autoradiographs of three gels showing the sequence of the G4 gene *K* region are shown in Fig. 1. Because of the high homology between the ΦX174 and G4 DNA sequences in this region it was possible to use the ΦX174 *Taq* I fragment 9 as a primer with G4 viral or complementary DNA strand using the chain termination method (Figs 1a and 1c, respectively). This fragment extends from nucleotide 61 to 93 (Fig. 2). The *Taq* I site (T CGA) at the 5' end of this fragment is conserved in G4 but the site at the 3'

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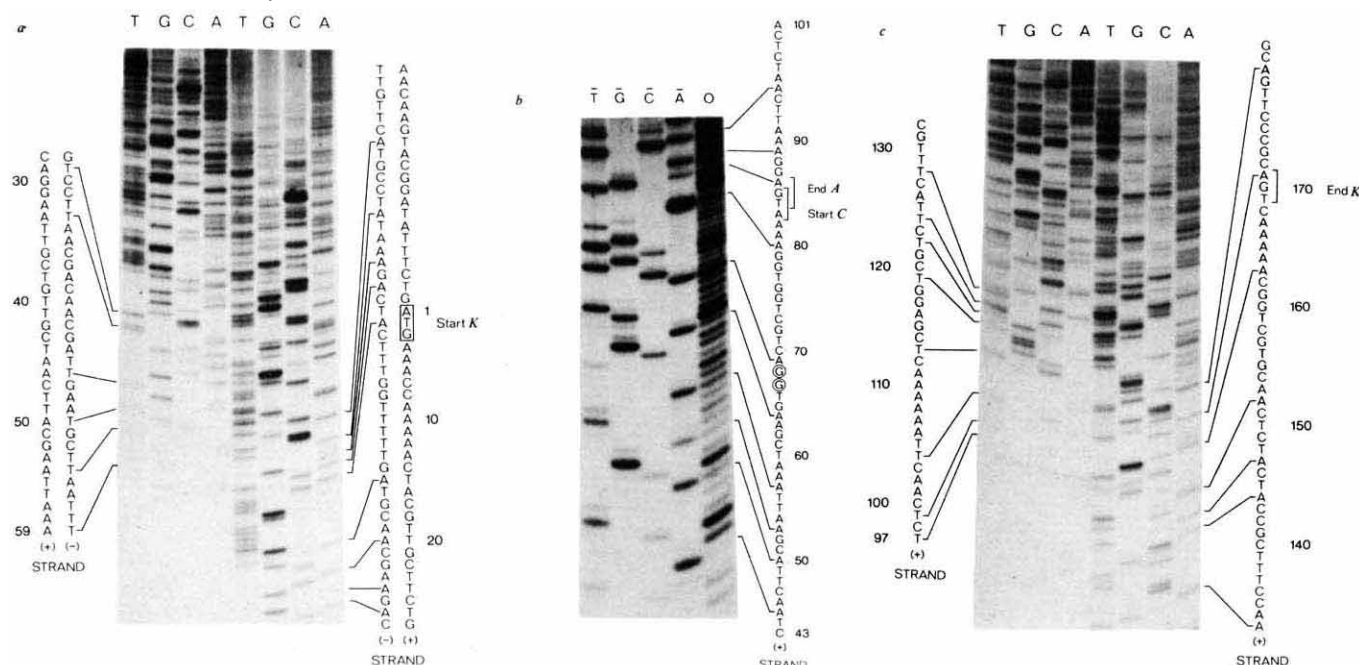


Fig. 1 DNA sequence analysis of bacteriophage G4 gene K. *a*, Phage Φ 174 *Taq* I fragment 9 primed on bacteriophage G4 viral strand using the chain termination procedure of Sanger *et al.*⁸. The products were not cleaved with restriction enzyme before fractionation on 8% polyacrylamide gels containing 7 M urea and Tris-borate-EDTA buffer, pH 8.3. The gel was loaded at different times; the four samples on the right were run for 3.5 h and the four samples on the left for 7 h at a constant current of 40 mA. The sequence deduced is that of the complementary strand and the viral strand (+ strand) is shown alongside. The sequence is numbered from the A of the ATG initiation codon. *b*, Phage G4 *Hpa* II fragment C2 was cleaved with *Pst* I and the ~580 nucleotide subfragment primed on bacteriophage G4 complementary strand (-strand) using the minus method of Sanger and Coulson²¹. The products were cleaved with *Pst* I before fractionation on 12% polyacrylamide using the same buffer as in (*a*). The sequence deduced is that of the viral (-) strand. The two G residues at positions 67 and 68 (circled) were obtained from other similar experiments (data not shown). *c*, As (*a*) but using phage G4 complementary strand as template. The deduced viral strand sequence is displayed alongside.

end is replaced by the sequence TCAA. However, there is enough homology preceding this sequence for this fragment to be used as a primer with G4 complementary strand template (Fig. 1c). Using this fragment the sequence from the proposed ribosome recognition site through to nucleotide 59 (Fig. 1a) and from nucleotide 97 to the termination codon (Fig. 1c) in gene K was obtained. The sequence in the middle of gene K was obtained using a *Pst* I/*Hpa* II fragment of bacteriophage G4 as a primer in the 'plus and minus' method with complementary strand template. This fragment extends approximately 580 nucleotides upstream from the bacteriophage G4 *Pst* I site (CTGCA ↓ G) at nucleotide 29 in gene K. The products of the primed synthesis reaction were cleaved with *Pst* I before gel electrophoresis. Figure 1b shows the minus system only which allows the sequence to be deduced from position 43 to 101 in gene K.

Thus the bacteriophage G4 sequence corresponding to the gene K region in bacteriophage Φ X174 was obtained and a comparison of the two sequences is shown in Fig. 2. This shows that the proposed ribosome recognition site (GGA) and the initiation (ATG) and termination (TGA) codons are conserved in the same positions in the nucleotide sequence. Comparison of the proposed coding sequences shows that approximately 66% of the amino acids are conserved and in particular the hydrophobic amino acids in the amino-terminal region of the protein.

Chemical identification and expression of gene K in G4

A comparison by polyacrylamide gel electrophoresis of the proteins produced by bacteriophages G4 and Φ X174 in infected *E. coli* cells shows an overall similarity (Fig. 3a). Proteins specified by Φ X174 cistrons A, B, D, F, G and H have been identified by comparison with proteins synthesised by Φ X174 amber chain termination mutants (for a review see ref. 9). In addition, protein chemical evidence has been obtained by classical means for proteins D (ref. 4), J (ref. 29),

Table 1 Amino acid composition of G4 protein K compared with amino acid compositions of proteins J and K derived from the G4 nucleotide sequence

Amino acid	Analysis of band K	From DNA sequence K	From DNA sequence J
Aspartic acid*	5.0	4	0
Threonine	3.0	3	1
Serine	3.8	5	3
Glutamic acid	9.3	10	1
Proline	2.0	2	0
Glycine	2.6	2	5
Alanine	1.8	1	1
Cysteine	ND	1	0
Valine	5.8	3	1
Methionine	1.2	1	1
Isoleucine	2.4	1	1
Leucine	12.5	15	1
Tyrosine	1.1	1	2
Phenylalanine	0.3	0	0
Histidine	0	0	0
Lysine	5.0	5	4
Arginine	2.0	2	3
Tryptophan	ND	0	1
Total	—	56	25

The protein was isolated by polyacrylamide gel electrophoresis in the presence of salt (Fig. 3b). Analysis was performed on acetone soluble material (75% of total) containing 10,000 c.p.m. eluted from leading band K in the presence of 0.2 mg horse heart cytochrome c. The material was hydrolysed at 110 °C with 6 M hydrochloric acid for 22 h and then analysed after the addition of 2 nM each of non-radioactive protein amino acids in a Durrum D-500 amino acid analyser modified for peak collection²³. The radioactivity eluted in each peak was measured²³ and then divided by the counts per residue of each amino acid incorporated into proteins by G4 in the experimental conditions used (see legend to Fig. 4). ND, not determined.

*Total of asparagine and aspartic acid residues. Only asparagine residues are found in protein K.

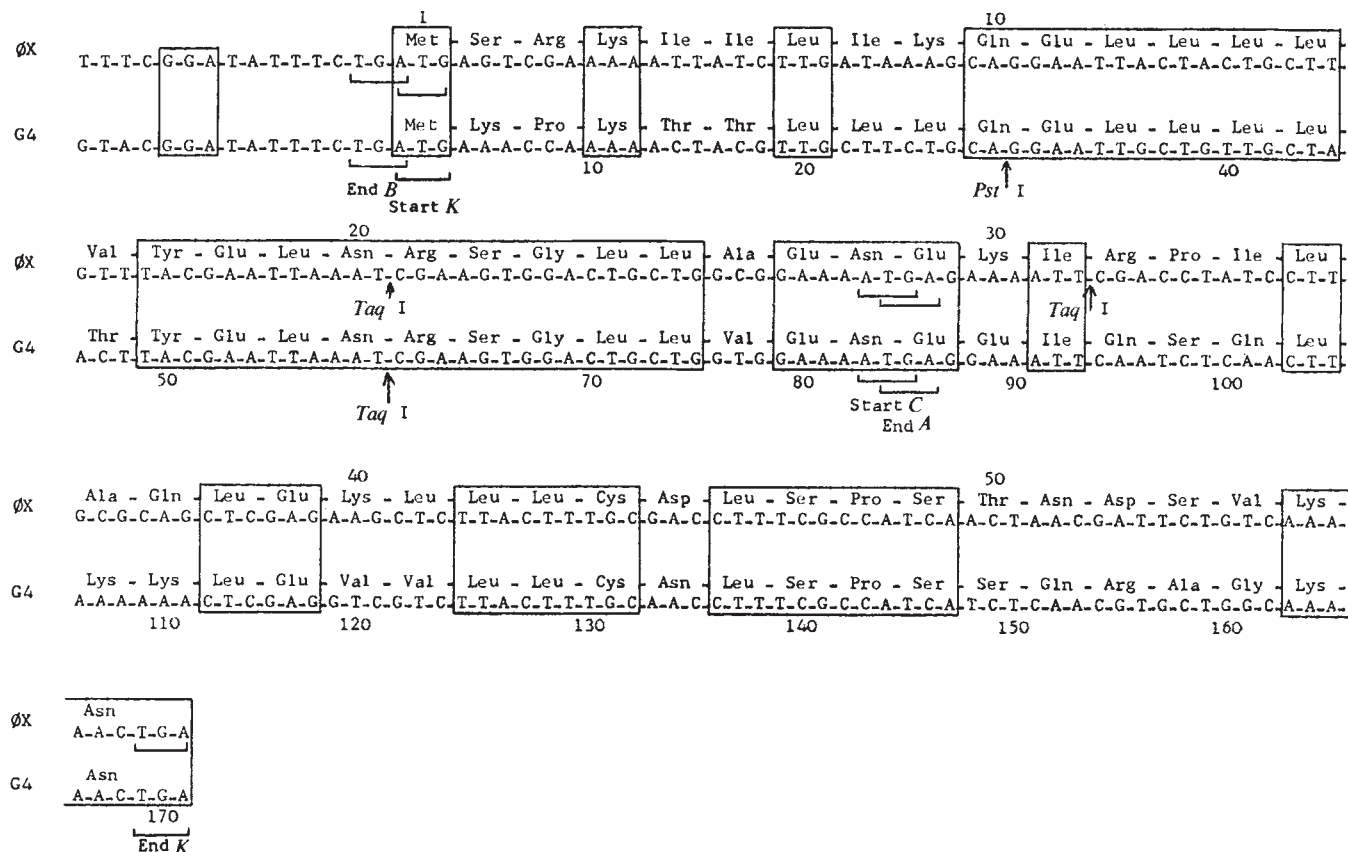
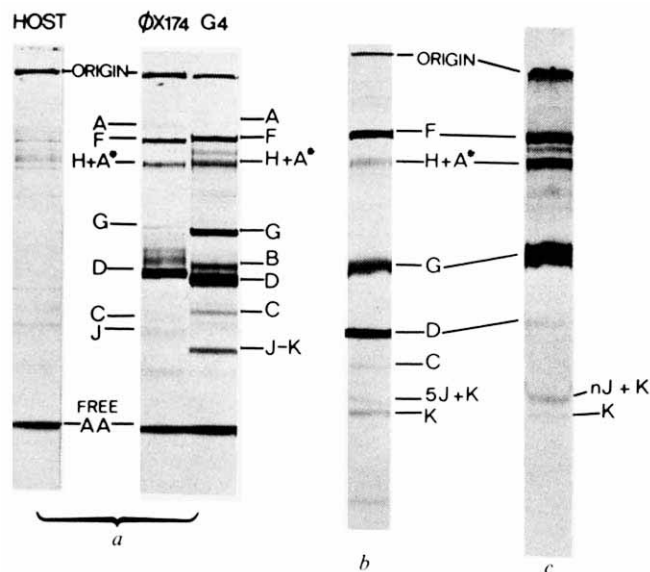


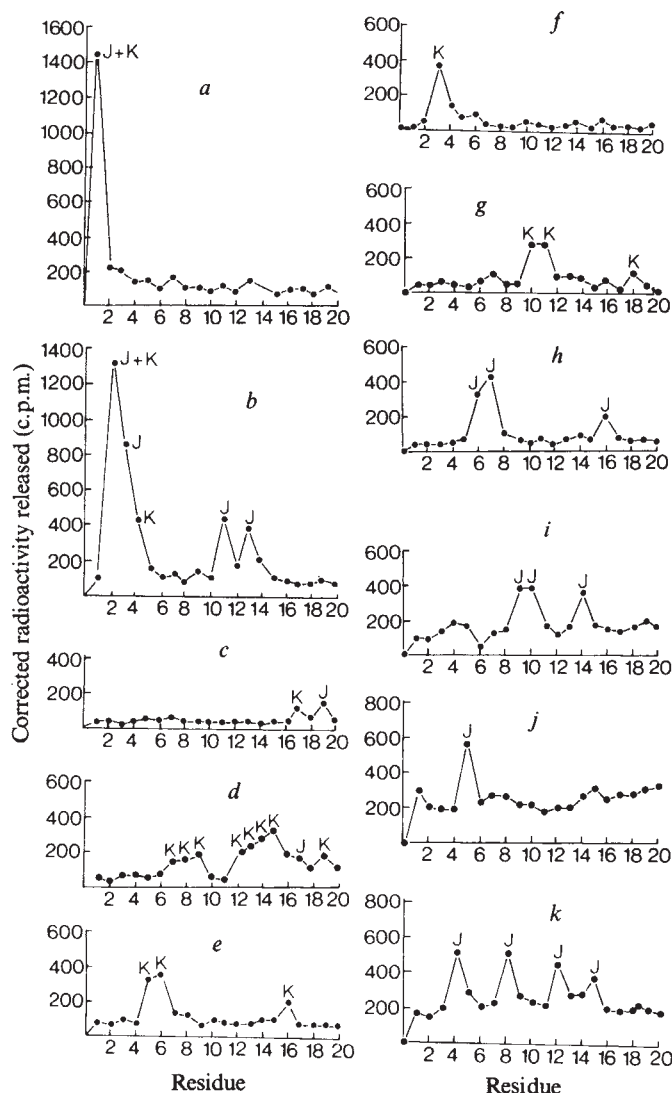
Fig. 2 Comparison of the DNA and protein sequence sequences of gene *K* from phages ΦX174 and G4. The ribosome recognition sequences are shown boxed along with homologous amino acids. The termination codons of gene *B* and gene *A* and the initiation codon of gene *C* are also indicated. The top numbering system refers to the amino acid residue number starting at the N-terminal methionine, and the lower numbering system refers to the nucleotide sequence starting at the A of the ATG initiation codon.

F (refs 10, 11), H (G. M. Air, unpublished) and G (ref. 12). The analogous bands in G4 have now been shown to correspond to the same cistron products as in ΦX174 by DNA sequencing (J.C.F., B.G.B. and G.N.G., unpublished) and direct protein microsequence analysis of eluted bands (D.C.S. and J.E.W., unpublished).

The most striking difference between ΦX174 and G4 proteins is a strong band labelled J+K in G4 which is absent from ΦX174 (Fig. 3). A possible candidate for this band was the small basic structural protein J which in G4 contains 25 amino acids (J.C.F., B.G.B., G.N.G., J.E.W. and D.C.S., unpublished). However, incorporation experiments with single radioactive

Fig. 3 Proteins of phages ΦX174 and G4. Autoradiograph of polyacrylamide slab gels after electrophoretic separation of radiolabelled proteins in the presence of SDS²². In *a*, proteins were isolated from a 2 ml culture, OD₅₀₀ 0.5, of phage infected ultraviolet irradiated cells of *E. coli* 4704 hcr⁻ Thy pulse labelled with ¹⁴C-amino acids¹. An algal hydrolysate (Amersham, UK) containing 150 μCi of amino acids supplemented with 10 μCi each of ¹⁴C-methionine, histidine, asparagine and glutamine was used for this purpose. Cells were centrifuged and the pellet heated at 100 °C for 3–5 min with sample buffer²² containing 8 M urea. The extract was applied to a polyacrylamide gel containing 15% acrylamide and 0.4% bisacrylamide but with no stacking gel. *a*, Host proteins derived from uninfected irradiated cells compared with proteins from ΦX174 and G4. *b*, Proteins were isolated from non-irradiated cells. Incubation in the presence of ¹⁴C-amino acids was continued to lysis (35 min). Debris was centrifuged in the presence of 10% w/v 6000 polyethylene glycol and 1 M sodium chloride. 10 μl of the pellet was dissolved in 100 μl of sample buffer containing urea and 20 μl applied to the gel. *c*, The remainder of the pellet in part *b* was fractionated by centrifugation (86,000g, 20 h, 18 °C) in neutral CsCl (1.40 g ml⁻¹). The virion band (density = 1.42 g ml⁻¹) was recentrifuged under the same conditions. CsCl was removed by dialysis for 3 h against 1% ammonium bicarbonate solution. The sample was lyophilised, redissolved in sample buffer (100 μl) and 20 μl applied to the gel. Some non-virion protein D was still evident on the gel and so it was not possible to state whether the K observed was within the virion or due to the incomplete separation from the soluble proteins. Bands F, B, D and C in G4 were identified as the proteins corresponding to these genes by direct microsequence analysis of the bands (D.C.S. and J.E.W., unpublished work). 5J+K implies that the ratio of J : K as determined by amino acid analysis was 5 : 1 and nJ+K that the ratio was not measured.





amino acids were not consistent with this view. For example, the ratio of intensities that would be predicted from the amino acid composition of J for incorporation of arginine and leucine separately is 3:1. A ratio of 1:3 was observed (D.C.S., unpublished). Protein microsequence analysis of material eluted from band J+K showed that it contained two proteins (Fig. 4). One corresponded to the G4 cistron J and the other to gene K. The amino acid sequences of these proteins predicted from the DNA sequence indicate that glycine, arginine, isoleucine, serine and alanine are all absent from the amino-terminal 20 residues of protein K. The release of radioactivity corresponding to glycine at steps 9, 10 and 14, of arginine at steps 6, 7 and 16, of isoleucine at step 5 and of alanine (see legend to Fig. 4) at steps 4, 8, 12 and 15, is entirely consistent with the predicted sequence of protein J. Similarly, threonine, proline and glutamic acid/glutamine are predicted as being absent from the first 20 residues of protein J. The release of threonine at steps 5, 7 and 16, of proline at step 3 and of glutamic acid at steps 5, 7 and 16 fits the predicted sequence of protein K. A remarkable feature of protein K is the presence of two runs of leucine residues at positions 7-9 and 12-15. The pattern of radioactivity released in the position of leucine corresponds to these sequences and in addition confirms a leucine residue at position 17 in protein J. The patterns of release of radioactivity corresponding to methionine, lysine and tyrosine also fit the predicted sequences of proteins J and K. In addition, consideration of the quantities of radioactivity at steps 2 (J+K), 3 (J alone) and 4 (K alone) shows that the mixture contains twice as much J as K.

It was subsequently observed that band J+K could be resolved into two bands by the addition of salt to the sample before application to the polyacrylamide gel (Fig. 3b, c). The amino

Fig. 4 Protein microsequence analysis of band J+K from bacteriophage G4. Material was recovered from 10 slots of a polyacrylamide gel similar to the slot shown for G4 in Fig. 3a. Elution was carried out in the presence of 2 mg of haemoglobin carrier as described in detail elsewhere²³. Protein was precipitated with 90% acetone containing 0.01% 12 N HCl. 60% of the radioactivity was precipitated by this procedure. The precipitated material containing 80,000 c.p.m. was applied to a spinning cup sequencer along with 2 mg of polybrene (hexadimethrinebromide)²⁴ and subjected to automated Edman degradation using dimethylallylamine buffer. Anilinothiazolones were hydrolysed to amino acids²⁵ in the presence of ¹⁴C-β-alanine as internal standard²⁶ and the radioactive amino acids released by the Edman degradation identified and quantitated with the aid of a Durrum D-500 analyser modified for peak collection^{23,26}. The recoveries of amino acids were corrected for hydrolytic and manipulative losses and for the different radioactivities of each amino acid as incorporated into the protein, with the aid of a computer program²³. For this purpose the specific radioactivities of amino acids were determined by radioactive amino acid analysis of protein D of G4 of which the amino acid composition was derived independently from nucleotide sequence analysis of gene D (J.C.F., B.G.B., and G.N.G., unpublished work). The panels show the release of radioactive amino acids during 20 cycles of the Edman degradation. a, Methionine; b, lysine; c, tyrosine; d, leucine; e, threonine; f, proline; g, glutamic acid; h, arginine; i, glycine; j, isoleucine; k, alanine. J and K denote amino acids consistent with the sequences (from DNA) of proteins J and K, respectively, which are as follows (see ref. 27 for notation)

	5	10	15	20
J	M	K	S	I
K	M	K	P	K
	T	L	L	L
	Q	E	L	L
	T	Y	E	L
	N			

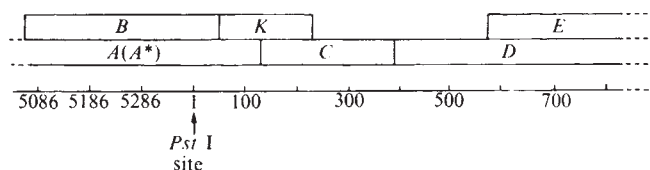
The identification procedure used does not allow the following pairs of amino acids to be distinguished: serine/alanine, glutamine/glutamic acid, asparagine/aspartic acid. Threonine is recovered as α-aminobutyric acid, isoleucine as isoleucine plus allo-isoleucine, and tryptophan as glycine plus alanine. No radioactivity corresponding to histidine or phenylalanine, both of which are absent from the first 20 residues of both proteins, was detected. No radioactivity attributable to valine-20 of J was found. ¹⁴C-Tryptophan was not present in the amino acid mixture used for pulse labelling, and hence residue 18 of J was not detected. An increase in aspartic acid at position 20 corresponding to asparagine-20 of K was noted.

acid composition of the leading band (Table 1), as determined by radioactive amino acid analysis, corresponds closely to the amino acid composition of protein K predicted from the nucleotide sequence. Further, it is consistent with the view that protein K as isolated from bacteriophage G4 by polyacrylamide electrophoresis corresponds to the complete translation product of gene K. The slower migrating band was a mixture of J and K in a ratio of 5:1. Radioactive amino acid analysis of acetone-precipitable and non-precipitable material derived from band J+K indicates that J and K proteins are present in G4 infected cells in equimolar quantities.

Genetic evidence for gene K in ΦX174

The high conservation of the protein K nucleotide and amino acid sequences in ΦX174 and G4 strongly suggests that ΦX174 also synthesises a K protein, but so far the ΦX174 protein has

Fig. 5 Position of gene K in the genetic map of phages G4 and ΦX174. A portion of the genetic map is shown in the overlapping gene region. The numbering system is that of phage ΦX174 (ref. 2 and F. Sanger, unpublished results), which is numbered from the single *Pst* I site. The single *Pst* I site in G4 is further downstream in gene K. Protein A* arises from a reinitiation of translation within gene A in the same translational reading frame²⁸.



Protein		Ref.
Protein K, G4	M K P K T T <u>L L L</u> Q E <u>L L L L</u> T Y E L N	This work
Protein K, ΦX174	M S R K <u>I I L I</u> K Q E <u>L L L L</u> V Y E L N	2
Protein E, G4	M V R W T L W N T L A F <u>L L L L</u> S <u>L L L</u> P S	*
Protein E, ΦX174	M V R W T L W D T L A F <u>L L L L</u> S <u>L L L</u> P S	4
MOPC 41 α -L-chain	M D M R A P A Q I F G F <u>L L L L</u> F P G T R L	18
MOPC 321 α -L-chain	M - - - <u>L L L</u> - - <u>L L L</u> - - P	18
Lactogen precursor	M P - - - - <u>L L L</u> - - - <u>L L - L</u> - P	19
Preproalbumin	M - - - - F <u>L L L L</u> F - - - - F	20
Cytochrome P-450	M E F S <u>L L L L L</u> A F L A G <u>L L L L</u> L F R G	16

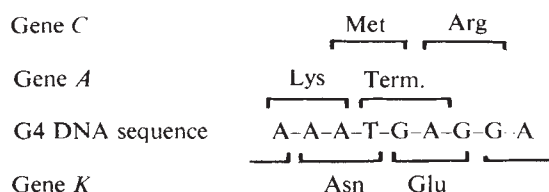
Fig. 6 Comparison of the amino-terminal sequences of proteins K and E of bacteriophages G4 and ΦX174 with the signal peptides of some secretory proteins and a membrane protein. The one-letter code for amino acids is given in ref. 27. *, J.C.F., B.G.B. and G.N.G., unpublished.

not been identified. However, there may be some genetic evidence for its existence in ΦX174. Gene *J* in ΦX174 was at first mapped between genes *D* and *E* and gene *F* on the basis of the amber mutant *am6* (ref. 13). This mutant was originally assigned to gene *E* (the lysis gene) on the grounds that it was lysis-defective¹⁴. However, because a low molecular weight protein (assumed to be the gene *J* product) was not detected in extracts of ΦX174-infected cells, the mutant *am6* was assigned to gene *J*. Genetic crosses tentatively located *am6* as being downstream of *am27* (*E*) and upstream of *op6* (*F*), although *am3* (*E*) and *am6* (*J*) could not be resolved¹³. DNA sequence analysis^{2,4} and amino acid sequence analysis of protein *J* has shown that gene *J* is located between the overlapping genes *D* and *E* and gene *F*.

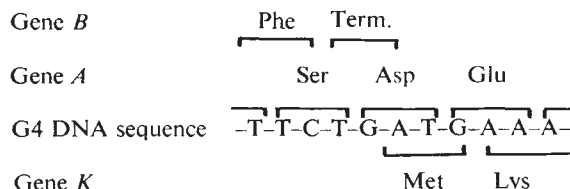
Recently *am6* has been shown to be a double amber mutant mapping in gene *A* and gene *E* (ref. 15). DNA sequence analysis of *am6* viral strand³ has shown that the mutation in gene *E* is the same as *am3* (ref. 4) and the amber mutation in gene *A* occurs 17 nucleotides before the end of gene *A*. Also found were a third position arginine codon change (A → G) 31 nucleotides before the end of gene *A*, and a missense mutation in gene *A*, TCG (Ser) to TTG (Leu), 77 nucleotides before the end of gene *A* (B.G.B., unpublished). This missense mutation in gene *A* would produce an *opal* (TGA) in the gene *K* reading frame in place of CGA (Arg). Thus it may be that gene *J* was fortuitously localised between *D/E* and *F* and that the low molecular weight protein observed to be missing in *am6*-infected cells was the gene *K* protein. This view is supported by the fact that DNA sequence analysis of 50% of gene *J* from ΦX *am6* has not yet revealed any mutation (B.G.B., unpublished).

Five of the protein K nucleotides are read in all three translational phases

A linear map of the overlapping gene region of ΦX174 and G4 is shown in Fig. 5. Genes *A*, *B*, *C*, *D* and *E* are in the same relative position in both ΦX174 and G4, and in both bacteriophages the termination and initiation codons of genes *B* and *K* overlap by one nucleotide (TGATG) and of genes *A* and *C* by two nucleotides (ATGA). This results in the A nucleotide of the gene *B/K* overlap (TGATG) being read in three translational phases—as part of an Asp codon in the A phase, a Met codon in the K phase, and as part of the TGA termination codon in the B phase, that is



In the cistron *A/C* overlap (ATGA) four nucleotides are read in all three translational phases and it is noteworthy that one nucleotide is recognised by three different tRNAs for the three genes *A*, *C* and *K*. In addition, the termination codon of gene *A* is also used in two other reading frames for genes *C* and *K* that is



Function of protein K

Although relatively large amounts of protein K are present in the lysate of G4-infected cells no function has yet been ascribed to it. Like protein E, the lysis protein of G4 and ΦX174, protein K has two stretches of hydrophobic residues close to its amino terminus. Similar features have been noted in a membrane protein, cytochrome P450 from rabbit liver microsomes¹⁶, and in precursors of secretory proteins¹⁷⁻²⁰. A comparison of the sequences of protein K and E from ΦX174 and G4 with some of these proteins is shown in Fig. 6 which illustrates this similarity. A membrane associated function has been suggested for the hydrophobic amino-terminal extensions of secretory proteins during protein synthesis of the polypeptide chain on membrane bound polyribosomes. These so-called signal peptides are subsequently processed off¹⁷. Although there is no evidence for processing of protein K the presence of hydrophobic features taken with similar features in the lysis protein suggests a membrane associated function. It is not yet known whether protein K is packaged in the virion (see legend to Fig. 3c).

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letters to nature

Clumpy structure of the Universe and general field

OBSERVATIONS of redshifts on samples complete to a given limiting magnitude have shown that redshifts are segregated or, in other words, that ranges of velocities exist in which for a given region of the sky no galaxies are observed¹⁻⁹. Such observations could fit the empirical model briefly discussed by myself and Rood³. We suggested that visible matter in the Universe is found in groups and/or large asymmetric structures, superclusters. Empty, or nearly empty, regions are observed in between. Such an empirical model was confirmed by data obtained in the Hercules region. The model may agree with the theory and numerical analysis developed by Zeldovich and Coll¹⁰. This note stresses that the observations set a strong limit on the density of the so called 'field galaxies' and questions the very existence of such objects.

Such research is parallel to the study of the distribution of galaxies; counts in which large inhomogeneities and fairly empty regions are observed. It is also closely related to correlation function arguments of Fall *et al.*¹¹ and Soneira and Peebles¹². I emphasise here the significance of the gaps or holes that are observed in the redshifts distribution, I will use the derivation suggested by Fall (personal communication) as it is more straightforward than mine (see also Fall and Tremaine, ref. 13). The expected number of galaxies with velocities in the range (V_1 , V_2) in a sample which is magnitude limited at m_0 is, assuming an uniform distribution,

$$N(V_1, V_2) = \Delta\Omega \int_{V_1/H}^{V_2/H} x^2 \phi(x) dx$$

where

$$\phi(x) = \bigoplus [M = m_0 - 5 \log(x/\text{Mpc}) - 25]$$

and

$$\bigoplus(M) = A \begin{cases} \text{dex}[\beta(M - M^*)], & (M \geq M^*) \\ \text{dex}[\alpha(M - M^*)], & (M < M^*) \end{cases}$$

$$(M^*_{\text{pg}} \simeq -18.6 + 5 \log(H/100); \alpha \simeq 0.75; \beta \simeq 0.25)$$

With

$$D^* \equiv \text{dex}[\frac{1}{5}(m_0 - M^*) - 5] \text{ Mpc}$$

and

$$V^* \equiv HD^*$$

we obtain for $V_1 < V^* < V_2$

$$N(V_1, V_2) = \Delta\Omega AD^* \{ (3-5\beta)^{-1} [1 - (V_1/V^*)^{3-5\beta}] + (3-5\alpha)^{-1} [(V_2/V^*)^{3-5\alpha} - 1] \} \quad (1)$$

however, the mean surface density of sample galaxies is given by

$$\frac{N(0, \infty)}{\Delta\Omega} = 1.905 AD^* = C 10^{0.6m_0} \quad (2)$$

Using the Zwicky catalogue and $m_0 = 15.7$ (ref. 14), we obtain

$$\frac{N(0, \infty)}{\Delta\Omega} \simeq 4.83 \times 10^3 \text{ str}^{-1}; D^*_{15.7} = 72.4(H/100)^{-1} \text{ Mpc}$$

and

$$A \simeq 6.68 \times 10^{-3} (H/100)^3 \text{ Mpc}^{-3}$$

If we apply this reasoning to the preliminary data of the Hercules cluster (Tarenghi *et al.*⁷) we can compute the number of field galaxies we would expect to observe in the gap between $V_1 = 5,500 \text{ km s}^{-1}$ and $V_2 = 8,500 \text{ km s}^{-1}$, Fig. 1. The sample is almost complete in the surveyed region, $\Delta\Omega \simeq 7 \times 10^{-3} \text{ str}^{-1}$. Using equation (1) and $V^* \equiv HD^* \simeq 7,240$

$$N(V_1, V_2) \simeq 6.5$$

the gap would, therefore, appear to be significant.

The large majority of galaxies in the Zwicky catalogue belong, therefore, to clusters, superclusters or groups. Another way to look at this result is: if the mean surface density of the galaxies in

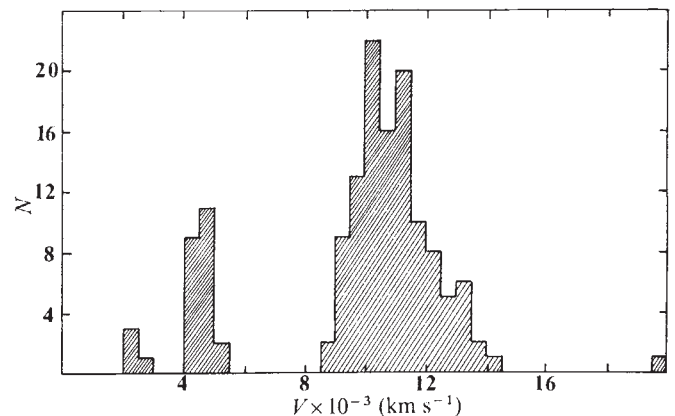


Fig. 1 Computed number of galaxies in the gap between $V_1(5,500 \text{ km s}^{-1})$ and $V_2(8,500 \text{ km s}^{-1})$.

Zwicky catalogue is due to a uniform field, we would have, according to equation (2)

$$N(< m) = 1.84 \times 10^{-6} 10^{0.6m}$$

Such a hypothetical field must be at least a factor 6.5 less dense, that is,

$$N(< m) \leq 2.8 \times 10^{-7} 10^{0.6m}$$

This result agrees with Soneira and Peebles¹², and Fall *et al.*¹¹.

I have tried to stress the simple and straightforward way of setting an upper limit for the density of the hypothetical uniform field of galaxies. It is, therefore, important to look for redshifts in rather large regions and observe fainter magnitudes to see if the gaps in redshifts remain and whether we keep finding non-uniformity both in the surface distribution and in depth.

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Joule dissipation in F-region and equatorial spread-F events

THE dissipation of turbulent electric fields varying with time, which are associated with spread-F in equatorial regions, has been thought to cause significant heating of the neutral atmospheric gases at F-region altitudes and higher¹. The time-varying electric fields have been shown to exist during spread-F at Jicamarca², and the two phenomena are known to occur with high probability in a latitudinal belt about 30° wide centred on the magnetic equator³. The theory of formation of large-scale field-aligned irregularities¹ from which small-scale irregularities, as in spread-F, may develop³ suggests that if spread-F occurs, it will happen all along the magnetic tube of force⁴. This inference is consistent with the observations³ of 'turbulent' electrostatic fields from satellite OVI-17. We describe here recent experimental measurements of neutral atmosphere temperatures at F-region heights at Mt Abu (24.6°N, 72.7°E geographic; 15.0° geomagnetic latitude) which agree with the theoretical prediction of very great atmospheric heating in equatorial spread-F events¹.

A digitally pressure-scanned Fabry-Perot spectrometer from the Physical Research Laboratory, Ahmedabad, was tested by measuring the Doppler width of 6,300 Å O(1D) airglow line during two nights in February 1976 at Mt Abu⁵. It was later improved and made more sensitive by using larger plates and controlling the instrument by a single frequency He-Ne laser line at 6,328 Å. It was possible to measure the Doppler width of the 6,300 Å line to an accuracy of 1.5 mÅ with this arrangement. This corresponds to an accuracy in temperature measurements to within ± 70 K. Table 1 shows the data obtained on different nights, and the neutral atmospheric temperatures obtained from these measurements. The geomagnetic

Table 1 Observed neutral atmosphere temperatures over Mt Abu, India

Time (IST)	21.2.76	22.2.76	9.10.77	11.10.77	12.10.77	13.10.77
1930	892†	918†	†	*	†	909*
2000	†	†	1,076†	*	850†	*
2030	1,296†	1,210*	862†	*	†	990*
2130	†	*	†	1,245*	†	†
2230	*	850*	†	*	†	†
2300	*	*	†	1,192†	†	†
2315	865*	825*	†	†	†	†
0030	†	900*	†	†	†	†
Σ K-index	23	22	9	18	28	20

All temperatures measured in K.

*Spread-F present over Thumba.

†Spread-F absent over Thumba.

‡No data available on spread-F.

activity represented by the sum of K-indices is also given for each day at the end of the table. It is found that neutral atmosphere temperatures are 200–300 K higher than those calculated, assuming the atmospheric model of Jacchia⁷ to account for the observed intensity of 6,300 Å oxygen line which was simultaneously recorded with a filter photometer at Mt Abu. In general, we found that the measured neutral atmospheric temperatures at the Mt Abu peak occur between 2000 and 2130 IST and do not seem to be directly associated with the geomagnetic activity. Spread-F activity, which normally starts on the equator after sunset, seems to be associated with these temperature increases. Unfortunately we do not have ionograms from Mt Abu, so the spread-F information is obtained from ionograms at Thumba, on the geomagnetic equator and in approximately the same meridian.

It is clear that the neutral atmospheric temperature at Mt Abu during equatorial spread-F occurrence at Thumba is 200–300 K higher than when there is no spread-F at Thumba and also higher than can be deduced from the accepted model atmosphere. These temperature enhancements imply a temperature gradient between the F and E region of the order of 1 K km⁻¹, the same as predicted by Cole. These observations are consistent with the model⁶ of Cole for formation of spread-F on large-scale field-aligned irregularities between conjugate dynamo regions of the ionosphere and the estimates of heating in the spread-F events¹. Thus, these results provide fairly unambiguous evidence that there is significant heating of the atmosphere above 300 km due to Joule dissipation of currents generated in the F-region by turbulent electric fields during spread-F events over and near the magnetic equator. A detailed paper on this topic will be published elsewhere.

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IMF effects on short-period fluctuations at low latitudes

THE influence of the north-south component (B_z) of the interplanetary magnetic field (IMF) on geomagnetic activity is well established^{1,2}, although a search for closer links between them has encountered some difficulties because magnetospheric responses depend on many parameters. It is also expected that the effects would be dominant at high or auroral latitudes. These studies are important because the B_z component controls the transfer of energy carried by the solar wind into the magnetosphere and consequently into the lower atmosphere, a phenomenon which has gained considerable importance in solar-terrestrial relationships. Here we investigate the effect of B_z variations at low latitudes particularly the equatorial region, which is relatively free from particle precipitation. Furthermore, due to the high electrical conductivity of the ionosphere during the daytime over this region, variations near the magnetic equator are very sensitive to changes in the electric field. Thus, equatorial stations may reveal some new features of the correlations between geomagnetic variations and the IMF.

As the variations in the equatorial region are highly time-dependent, pairs of low-latitude and equatorial stations have been chosen for analysis from widely separated longitudinal zones—American zone (Huancayo, Fuquene), African zone (16DIR, lat. 9.6°N, long. 41.87°E, dip. lat. 0.20°, a temporary station in Ethiopia) and the Indian zone (Trivandrum, Alibag). Huancayo (lat. 12.05°S, long. 284.65°E, dip. lat. 1.09°) and Trivandrum (lat. 8.48°N, long. 76.95°E, dip. lat. -0.45°) are the equatorial stations in their respective zones, and Fuquene (lat. 5.47°N, long.

286.27°E, dip. lat. 18.12°) and Alibag (lat. 18.63°N, long. 72.87°E, dip. lat. 12.70°) are low latitude stations.

For the present analysis, storm time variations of 9 April 1971 were chosen. Figure 1b shows variations of the horizontal component at the three equatorial stations during the storm and, the raw data from the IMP-I satellite, B and θ of the IMF. The storm time variations between 0000 UT and 2400 UT were digitised at 3 min intervals at each station. From the time series, so formed, diurnal trend, Dst and recovery variation were eliminated by applying a 61-point sine-terminated highpass filter designed by Behannon and Ness³. The filter has a unit response to all variations of period less than 100 min. The filtered series were subjected to a fast Fourier transform to obtain power spectra of H variations at all the stations. B_z , derived from IMP-I data, was similarly analysed. Figure 1a shows the power spectra of B_z and H at the three equatorial stations. For brevity, only the results obtained for the equatorial stations are shown here. The peaks in the spectra are clearly similar in all the traces, except for a few slight shifts in period.

The comparison of features in the power spectrum is not a satisfactory index for establishing correlations between B_z and magnetic variations. Power, as computed above, is a measure of the integrated amplitude of variations of the same period in the whole record. It does not make any distinction between fluctuations occurring in day and night hours, whereas the fluctuations near the dip equator are strongly enhanced near local noon. The enhancement also has a regional and period dependence. This is shown by the differences in power from station to station in Fig. 1a.

This limitation can be overcome by filtering the series with band-pass filters to isolate periodicities of interest, and then studying the response of that period with time. Three band-pass filters corresponding to the selected periods of 70, 48 and 21 min, each of 141 points were designed³.

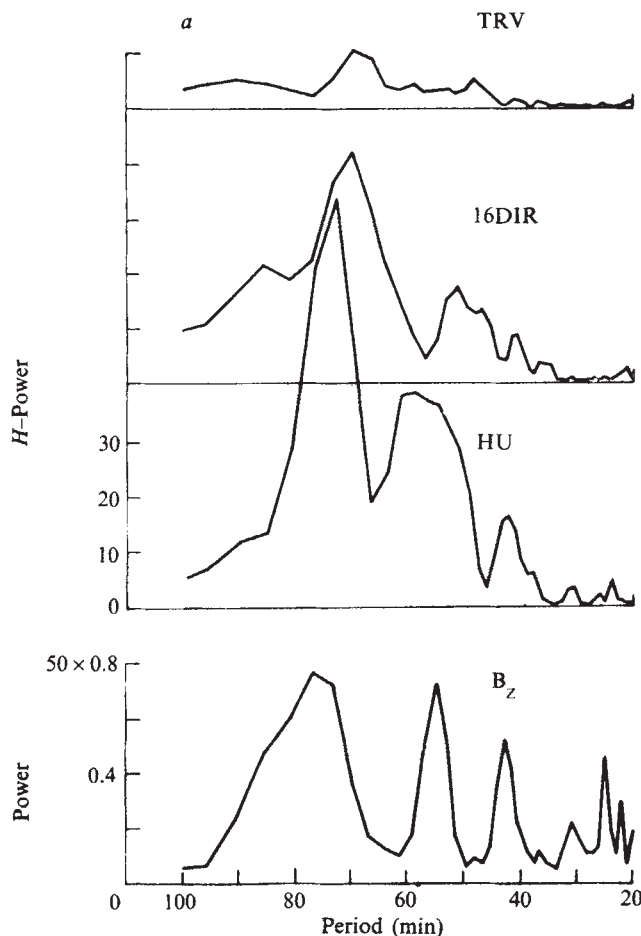
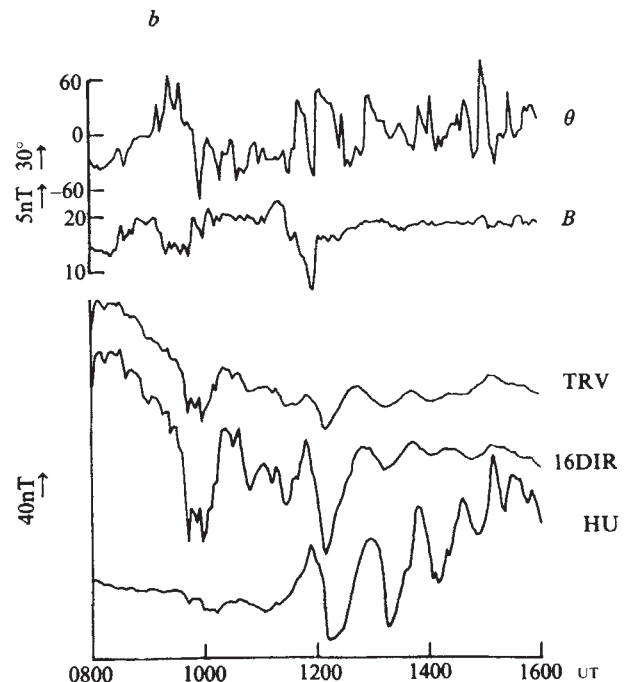


Fig. 1 a, Power spectra; b, traces of actual H variations at the three equatorial stations Huancayo (HU), Trivandrum (TRV) and 16DIR along with the power spectrum of B_z and IMP-I data.



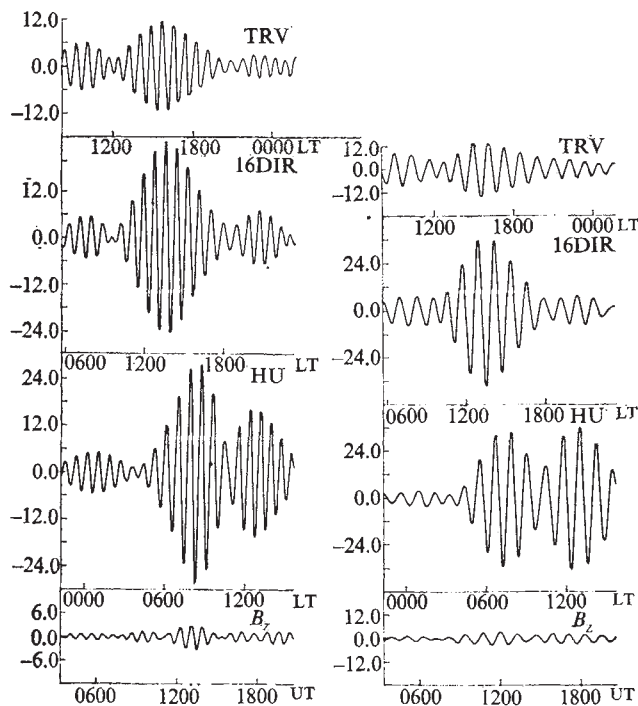


Fig. 2 The band-pass filtered series showing variations in amplitude of B_z and H at the three equatorial stations for oscillations of a , 48 min; b , 70 min period.

Maximum unit response was obtained for the central period of interest.

The striking common features obtained in the band-pass filtered series are necks or compressions in the B_z variations which are reflected at all three stations, irrespective of longitude. Figure 2a and b shows the series for the three stations and B_z for 48 and 70 min, respectively. This effect is equally present at the equatorial and low-latitude stations, which are not shown here. The envelope of amplitudes shows the expected enhancement during day hours. This is largest at Huancayo in the American zone, which has the greatest range of solar quiet day variations⁴. The maximum enhancement, however, is not at local noon, but is controlled by the amplitude of B_z , as demonstrated in Fig. 2b. The neck in B_z at about 1700 UT for 70 min oscillations is impressed on the variations at Huancayo despite it being local noon there. Similar necks are seen in the traces of Trivandrum and 16 DIR. 48-min oscillations have two necks at 0730 and 1530 UT at all stations, corresponding to similar signatures in B_z (Fig. 2a). Such a correspondence is also observed for 21-min oscillations but these traces are omitted for conciseness.

We conclude that there is a strong correlation between B_z variations and the fluctuations in magnetic fields in all regions and at all periods. Peak-to-peak amplitudes measured at each station at 1342 and 1706 UT for each of the three periods are given in Table 1. The figures suggest that the ratio of ground observations to B_z is largest for 48 min. This indicates that the magnetosphere allows interplanetary B_z oscillations around 50 min to propagate most easily down to the ionosphere. There is a case for thorough investigation of this period-dependence, under different conditions of activity. The influence of B_z , now established for short periods at low-latitudes, could be a good method of studying variations in the IMF.

The common features in these periods of oscillation which are observed on a global scale are very distinct signatures of the IMF. They can be the means of computing certain IMF parameters for intervals of time in the pre-satellite era.

Table 1 Peak-to-peak amplitudes of two moments

Time (UT)	Period (min)	B_z component of IMF (nT)	American region FN	HU	Indian region ALB	TRV	Ethiopian region 16DIR (nT)
1342	70	5	17	65	20	20	45
1342	48	6	12	56	16	13	34
1342	21	2	2	14	1	1	5
1706	70	6	15	70	11	9	14
1706	48	2	8	33	4	6	13
1706	21	2	1	3	1	1	1

Peak-to-peak amplitudes of oscillations of B_z and at three equatorial and two low-latitude stations for 70, 48 and 21 min are compared.

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Renewable energy sources and storage

A FEATURE of wind, wave and solar energy sources is the unpredictable variability of the strength of the source due to the vagaries of the climate. There are two main ways of exploiting these sources. In the first a back-up supply is provided. Thus, with solar water heating, it is customary to rely on electrical immersion heaters or boilers burning



Fig. 1 The eight sites where the hourly wind speed was recorded.

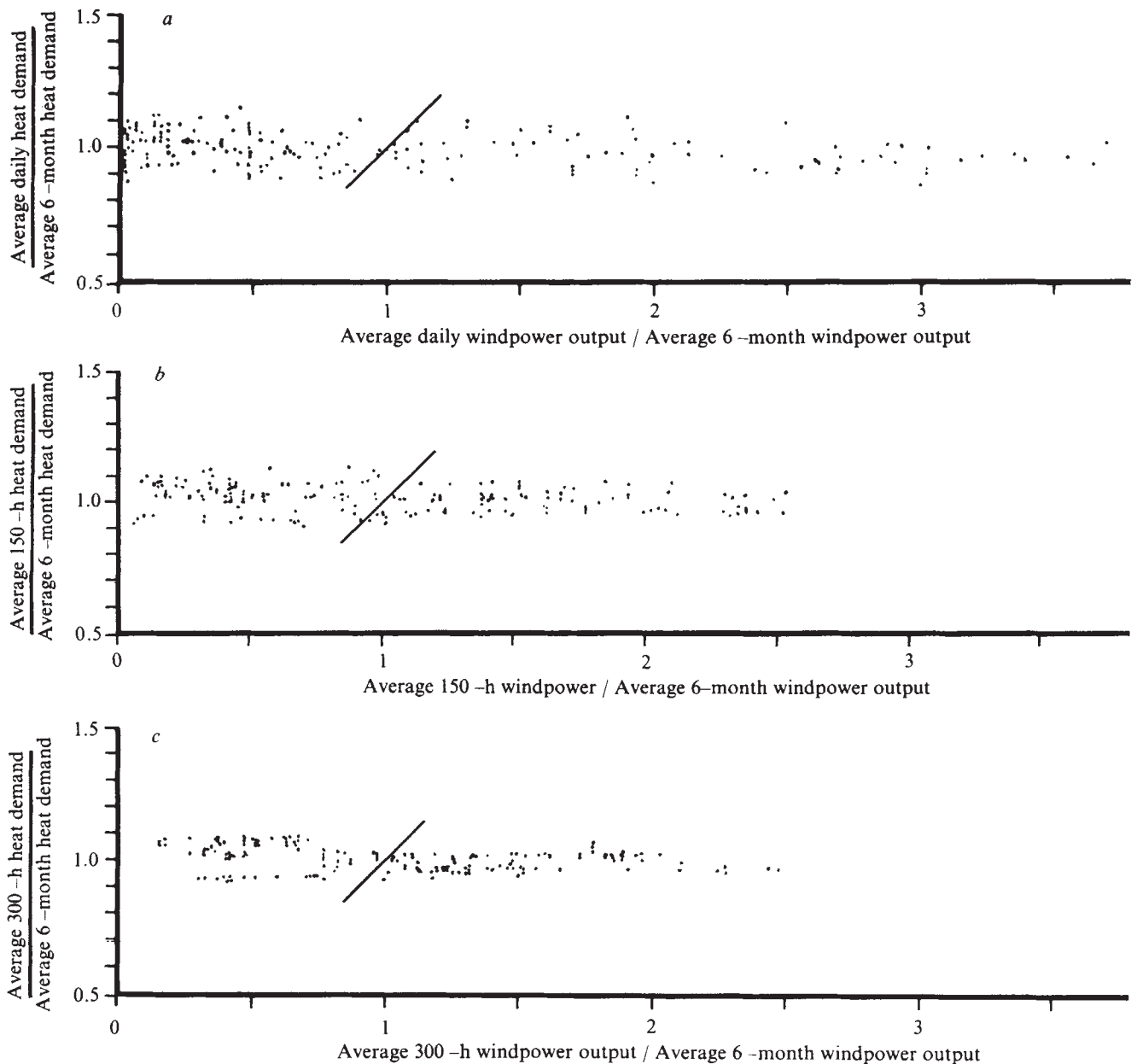


Fig. 2 Average heat demand plotted against average wind output. *a*, Daily; *b*, 150-h periods; *c*, 300-h periods.

gas, oil or solid fuel to supplement the solar energy input during the winter months and at other times when the insolation level is low. It is generally accepted that large wind generators would feed their electrical output into the national grid. On windy days the output would reduce the amount of electricity generated in conventional power stations, thereby saving fuel. The installation of a wind or solar power plant by itself, however, will save little, if any, conventional generating capacity, as there can be no guarantee of significant wind or solar generation at times of peak demand. The second way of exploiting renewable energy sources is to couple them with some form of storage. If the storage capacity is sufficient there is a guaranteed output from the renewable energy source plus storage system at the times when it is required. Ryle¹ has suggested that a form of storage is available in connection with wind power. We analyse here the validity of this claim.

In an electricity supply system, if the output can be guaranteed at times of peak demand there will be a saving

of conventional power station capacity as well as fuel. Provided the cost of storage is not too great, this improves the economics of renewable energy sources. For solar heating the peak demand is in winter, whereas the source is strongest in summer. This implies that storage would have to be sufficient for about six months, but it is likely to prove too expensive to be economic for UK climatic conditions. For wind and wave power both supply and demand peak in winter, hence seasonal storage is not required. Hydro-electric pumped storage and similar systems which regenerate electricity would not, however, be economic for the long storage periods needed. Ryle¹ has, therefore, suggested that the electrical demand for space and water heating could be met by wind power generation by providing a form of heat store on the consumers' premises with a capacity of 150 h (just under a week) and making the storage heating load disconnectable from the electricity supply system by a suitable form of remote switching. He suggests that large-scale wind generation would be significantly cheaper than

wave power or nuclear energy. His estimates seem to be based on very optimistic estimates of wind generator performance on average sites and his conclusion on relative economics is probably incorrect. There are several other errors (for example, in Table 1 the oil burnt in gas turbines is a factor of 60 too high) but this should not detract from the important suggestion that a cheap form of 150 h storage is feasible with wind power.

Hour-by-hour magnetic tape recordings of meteorological data were obtained for the eight sites shown in Fig. 1 between 1961 and 1976 and used as the basis for the analysis described below. The sites were chosen because they were geographically well spread and typical of the areas where windmill generators might be located. The wind speed data, after correction for the difference between the recording instrument height and the windmill rotor height, were fed into simulated wind generators having the following characteristics: rotor diameter, 60 m; rated wind speed, 14 m s^{-1} ; furling wind speed, 27 m s^{-1} . (Where rated wind speed is the lowest wind speed needed to generate maximum power output, and furling wind speed is the wind speed above which the rotor blades are turned into a position of zero output to protect the installation against excessively high winds.) For wind speed in m s^{-1} the generator output in kW was: 6.8 m s^{-1} , 0 kW; 7 m s^{-1} , 0.7 kW; 8 m s^{-1} , 32 kW; 9 m s^{-1} , 112 kW; 10 m s^{-1} , 261 kW; 11 m s^{-1} , 433 kW; 12 m s^{-1} , 617 kW; 13 m s^{-1} , 800 kW; $14\text{--}27 \text{ m s}^{-1}$, 1,000 kW; $>27 \text{ m s}^{-1}$, 0 kW.

Assuming a sufficient number of windmills, installed in equal numbers at each site, to provide the output needed to match the heating season consumption of an arbitrarily chosen number of all-electric households, the hourly and hence the daily outputs were calculated for the 16-yr period of records.

Empirically based relationships were used to calculate the corresponding hourly and daily total demand for water and space heating as a function of ambient temperature.

Figure 2a shows a plot of average daily heat demand against average daily wind energy output between 1 October 1974 and 31 March 1975, both being expressed in non-dimensional form by dividing by the average demand over the 16 years. As expected, there is little correlation between the two parameters. Whereas the standard deviation of the heating demand is only 0.062, that of the wind energy output is 0.984. Figure 2b shows the averages over 150-h periods, starting on successive days. If Ryle's contention were correct, we would expect to see the points lying close to a straight line through the origin of slope unity. This

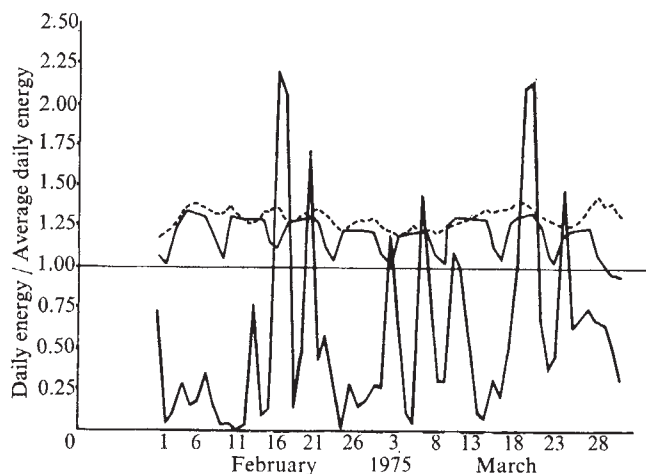


Fig. 3 Averages of the daily heating demand (dashed line), daily output of wind generation (thick line), and the CEGB demand (fine line) for the eight sites in Fig. 1.

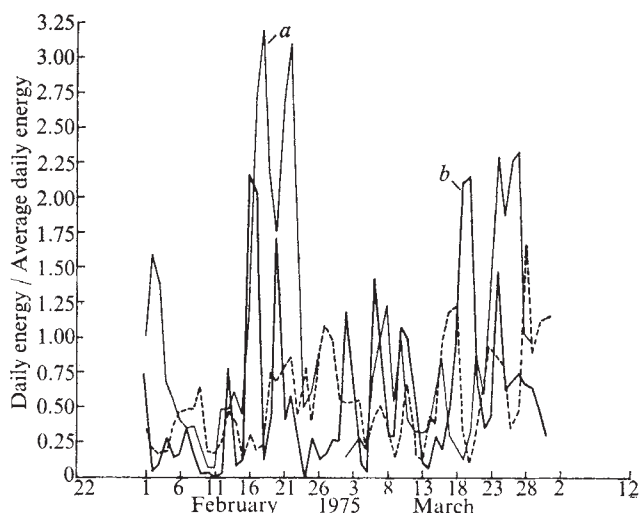


Fig. 4 Daily averages of energy from a, North Atlantic waves; b, wind energy, and solar energy (dashed line).

is far from the case. The standard deviation of the heating demand has fallen to 0.049 but that of the wind energy has only gone down to 0.673. Figure 2c shows the averaging taking place over 300-h periods. The standard deviation of the heating demand is now only 0.044 but that of the wind energy output has only fallen to 0.560.

The analysis can be clarified by considering a specific period of time—February and March 1975 were chosen because it is an extreme example. Figure 3 shows the calculated daily heating demand and the calculated daily output of the wind generators on the eight sites of Fig. 1, both expressed as a ratio of the long-term average of these quantities. As a check on the estimation of heating demand the total CEGB demand has also been plotted. The shapes of the two curves show reasonably close correspondence. Over this two month period, the heating and electricity demands are shown to be between 1 and 1.5 times the long-term average.

The mean wind energy available over the 59-d period is about 54% of the long-term average. A 150-h store, even if fully charged initially would have been emptied by 7 February. There would then be a shortage of energy for heating for a week until 16 February and further shortages from 21 February to 18 March. To cover this period satisfactorily a 38-d store, that was initially fully charged, would be required. It does not follow that a store of this size would be adequate for all periods, and further work is in progress to establish the relationship between size of store and firmness of power.

It is interesting to consider whether energy from the North Atlantic waves would arrive out of phase with the wind energy pattern and thereby provide some smoothing. The amount of information on wave energies over this period that we have been able to obtain gives only a partial coverage. The information is shown in Fig. 4; on the basis of this, it is clear that the availability of wave energy is correlated with that of wind energy so that using both sources would not greatly help the storage problem. Figure 4 also shows the daily average solar energy falling on a horizontal surface at Bracknell expressed as a ratio of the long-term average. It will be seen that there is a solar energy deficiency over the whole period.

The question of extent to which 'firm power' can be obtained from wind, wave and solar energy by incorporating cheap storage systems is complex and is being studied within the CEGB. However, sufficient work has already been done

to demonstrate that 150-h storage is quite inadequate to smooth the output of wind generators.

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New results from linearised charge transport theory

PROBLEMS involving the simultaneous transport of electrons and holes by drift and diffusion arise in connection with a great variety of solid-state phenomena and devices. Their analysis is handicapped because transport equations defy explicit solution for the general case. The choice must be made between computer-derived numerical solutions of the complete equations for particular parameters and boundary conditions, or explicit treatment of the equations with sufficient *ad hoc* assumptions (for example, bulk neutrality, zero recombination, freedom from traps) to permit their solution. The former is cumbersome and excessively specific for many purposes; the latter is often misleading, because the precise consequences of *ad hoc* assumptions cannot be easily foreseen. It is hoped that they affect only the accuracy of the results, but there are many instances when 'simplifying' assumptions distort the entire physical picture. We report here a third possibility, namely explicit solutions of the equations in linearised form, but without any other simplifying assumptions. The significance of these results is limited to low currents ('small signal theory') but the conclusions are otherwise general, and permit the convenient examination of the various processes as a function of the transport parameters. Moreover, the same set of equations can be applied to various problems by suitable adjustment of the boundary conditions. Such procedures have been used with two distinct problems:

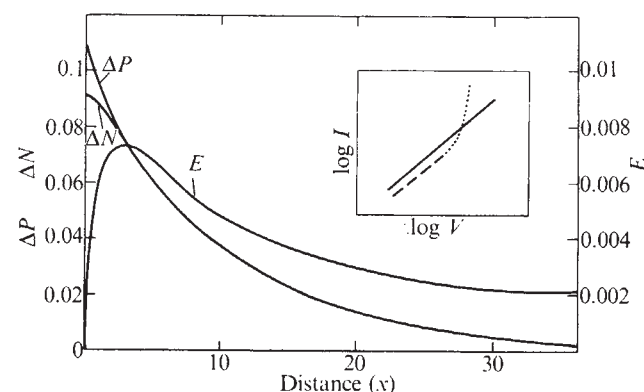


Fig. 1 Injection of holes (at $x = 0$) into an intrinsic lifetime semiconductor. Typical low current case for $\mu_n/\mu_p = b = 10$ and unit injection ratio. Distance x normalised to Debye length L_D , excess concentrations normalised to n_e , the equilibrium electron concentration. Field E normalised to kT/eL_D . Inset: solid line, unperturbed bulk; dashed line, present range, low current; dotted line, present range, high current.

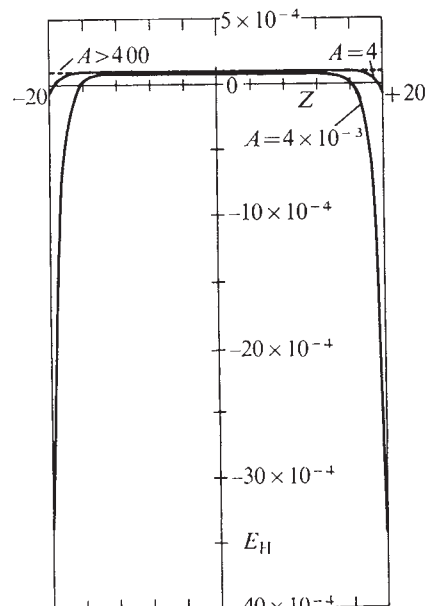


Fig. 2 Three Hall-field profiles. Distance Z in the direction of the Hall probes normalised to the Debye length $L_D = 1.02 \times 10^{-2}$ cm. E_{H0} = Hall field for very low lifetime. Hall fields normalised to kT/eL_D where $T = 300$ K. In these examples, dielectric constant = 10; $n_e = p_e = 6.9 \times 10^8 \text{ cm}^{-3}$; $\mu_n = 1,100 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$; $\mu_p = 1,000 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$; $s_n = 50 \text{ cm s}^{-1}$; $s_p = 100 \text{ cm s}^{-1}$ —low magnetic and longitudinal electric fields. $A > 400$; $\tau_o \leq 9.5 \times 10^{-9} \text{ s}$; $A = 4$, $\tau_o = 9.5 \times 10^{-7} \text{ s}$; $A = 4 \times 10^{-3}$, $\tau_o = 9.5 \times 10^{-7} \text{ s}$. Dashed line shows $E_H = E_{H0}$ where $A \geq 400$.

(1) the injection of minority carriers into lifetime and relaxation semiconductors, with and without traps^{1,2}, and (2) the Hall effect³ in specimens of finite size. The analysis even in linearised form is complex, and the details are published elsewhere, but explicit solutions can be obtained. They lead to new conclusions on the nature of the process involved.

It is usually assumed⁴ that the injection of minority carriers into a lifetime semiconductor (defined as one in which the diffusion length lifetime is greater than the dielectric relaxation time)⁵ reduces the total resistance of the system. This is true, but only if the current density is sufficiently high. For low current densities, however, a resistance increase may arise, associated with a field maximum. Its physical origin arises from the fact that the additional concentration of minority carriers (say, holes of concentration ΔP in an n-type semiconductor) attracts an almost equal concentration of majority carriers (electrons ΔN) into the vicinity, and thus sets up virtually equal concentration gradients $d(\Delta P)/dx$ and $d(\Delta N)/dx$. Of these, the former leads to a diffusion current which is additive to the field current, but the latter to one in the opposite direction. To compensate for this (and to maintain the total current constant) an extra field component is needed. On the other hand, the increased carrier concentrations imply a locally diminished field. When the electron mobility exceeds the hole mobility, the effect of concentration gradients can predominate over the effect of the augmented concentrations, and thus lead to a total resistance increase of the system¹. The conditions laid down by Kiess and Rose⁶ are fully maintained, as we are dealing with majority carrier augmentation, not depletion. As the current density increases, the bulk field increases also, and the localised field maximum tends to disappear. This implies a changeover from a total resistance increase to a total resistance decrease, as shown in Fig. 1. When the customary neutrality assumption is made on an *a priori* basis, the resistance increase disappears, which is presumably why it had not been reported before.

When the material is a relaxation semiconductor (defined as one in which the diffusion length lifetime is smaller than

the dielectric relaxation time)⁵, the situation is more complicated. In the trap-free case, the consequence of minority carrier injection is majority carrier depletion, associated with a resistance decrease, as Popescu and Henisch have shown⁷. When there are traps which capture predominantly minority carriers, the material can be driven into a 'lifetime regime' ($\Delta N > 0$) and can then also show the resistance increase⁸. The detailed consequences depend on many parameters, such as equilibrium carrier concentrations, mobility ratio, injection ratio, trapping parameters, and dielectric relaxation time.

Hall effect theory usually treats the specimen as infinite in the direction of the Hall field. An equivalent simplification is to assume that the carrier concentrations are everywhere in equilibrium (lifetime $\tau_0 = 0$). An alternative simplification is to assume $\tau_0 = \infty$ (ref. 9). Some attempts take into account^{10,11} the presence of surfaces characterised by variable surface recombination velocities. However, none of the previous solutions has been free from arbitrary assumptions, concerning neutrality, recombination conditions, dielectric relaxation time, and so on. Linearised transport theory, however, fits the analysis of this problem very well. It can be shown that $E_H = E_{H0} + \Delta E_{HS}$ where E_{H0} is the Hall field calculated in the conventional way ($\tau_0 = 0$, or specimen assumed infinite) and ΔE_{HS} a correction term dependent on specimen size and the nature of the surfaces, and the prevailing recombination statistics. Figure 2 gives a plausible set of parameters which shows how the Hall field can vary between the Hall electrodes, for three different lifetimes. The curves show that ΔE_{HS} can be comparable with E_{H0} and even exceed it in magnitude. Moreover, ΔE_{HS} can be of either sign, which means that geometrical, lifetime, and surface considerations can bring about a highly misleading reversal of Hall polarity. It is possible that the n-type Hall effect observed in high resistivity glasses¹² (which are otherwise p-type conducting) might be interpreted along these lines. This does not follow immediately from the present theory, because the model in its present form does not distinguish between recombination and trapping centres. However, such a distinction should be introduced and corresponds to observational necessities. Its consequences cannot be predicted with high reliability, but a more elaborate model which includes trapping as well as recombination will also multiply the circumstances under which Hall effect reversals can occur.

In a similar way, the linearised theory can be applied to systems under photoexcitation. Comparisons with computed solutions derived from the exact equations have shown complete agreement within the operational range delineated by the small signal theory.

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Copper-ion solid-state battery systems involving sulphonium iodide electrolytes

THE past 10 years has seen a considerable interest in solid-state battery systems involving electrolytes based on silver iodide¹⁻³. Studies of electrolytes involving organic sulphonium iodides in conjunction with silver iodide have been reported^{3,5-9}, but recently the possibility of using cuprous ions to replace silver ions as the mobile species has attracted attention, because of the potential advantage of lower cost and higher voltage. We report here the results of our investigations of sulphonium iodide-cuprous iodide electrolytes.

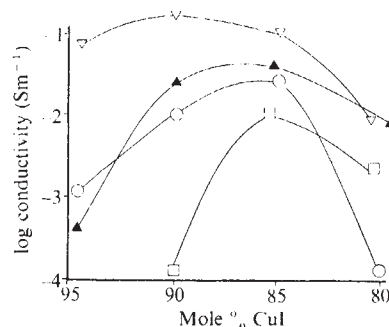
The sulphonium salts were prepared by adding the appropriate alkyl halide to an alcoholic solution of the parent sulphonium compound⁴. The empirical formula was determined by CHN analysis. Ultrapure cuprous iodide was used in all cases. The cuprous halide-sulphonium halide double salts were made by slurring the correct amounts together in an agate mortar, with a small amount of dry acetone, and drying and pressing into a pellet. The pellet was heated, under vacuum, to a temperature just below its decomposition temperature (determined by thermogravimetric analysis) for 48 h. Finally the pellet was reground and sieved through a 38 μ m mesh sieve. A three-layer disk was pressed, comprising 1 mm copper, 5-8 mm electrolyte, 1 mm copper, at a pressure of 350 N mm⁻². The initial conductivities, measured on a Wayne Kerr universal bridge type B221 A, were low in all cases but rose after 48 h to higher constant values.

In Fig. 1, the conductivities at 25°C of three electrolytes based on an iodide, and one electrolyte based on a bromide system are plotted as a function of mole percentage. Table 1 lists the maximum conductivities of these electrolytes, with our results and those of Takahashi *et al.*¹⁰ for four further mono-sulphonium cation electrolytes. Also listed are results for two test systems based on bis-sulphonium diiodides.

Table 1 shows that the highest conductivity was obtained for the electrolyte incorporating 4-methyl-1,4-oxathianium iodide, and this was therefore selected for the voltage tests reported below. We found its electronic conductivity, measured by the Wagner method, to be below 10⁻⁵ S m⁻¹; electronic conductivity measurements on the other electrolytes will be reported elsewhere.

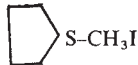
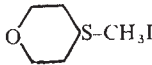
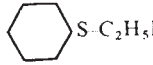
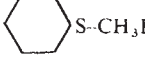
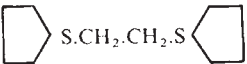
Note the different relationship between conductivity and sulphonium cation structure found for cuprous iodide systems, and for silver iodide systems: in the latter, the simple structural concept of organic cation group weighting coefficient (GWC) is useful for predicting which sulphonium cations form highly conducting electrolytes. This coefficient, described in detail elsewhere^{3,5}, takes account of size and shape in an empirical way, essentially by weighting the position of each carbon atom relative to the sulphur in the cation by a specific factor.

Fig. 1 The conductivities at 25°C of (C₂H₅)₃ SI (O); C₆H₁₀S C₂H₅I (▽); CH₃(CH₂)₆S(CH₃)₂I₂ (▲); (CH₃)₃ S Br (□) as a function of mole percentage.



This has a different value for chain, ring and bridge carbon atoms. A value of the GWC below a threshold of 7.5 corresponds to a high conductivity for the sulphonium electrolyte. For electrolytes involving cuprous iodide in place of silver iodide, no such simple threshold value of the GWC can be used. For example, in CuI systems, the electrolyte containing trimethyl sulphonium iodide has a very low GWC of 3.0; however, the conductivity is rather low and partly electronic. For the electrolytes based on CuI, thianium or oxathianium iodides have the highest conductivities.

Table 1 Electrical conductivities of copper (I) halide-sulphonium halide systems at 25 °C.

Sulphonium halide	Mole % CuI-sulphonium	Maximum conductivity (S m ⁻¹)	Decomposition Temp (°C)	Maximum conductivity by Takahashi (S m ⁻¹)
(CH ₃) ₃ SI trimethyl sulphonium iodide	86:14	* 1.3 × 10 ⁻²	143	
(C ₂ H ₅) ₃ SI triethyl sulphonium iodide	85:15	2.7 × 10 ⁻¹	84	
 1-methyl-1-thionia-cyclopentane iodide	85:15	* 4.2 × 10 ⁻¹	127	
 1-methyl-1-thionia-cyclohexane iodide	84:16	1.2 × 10 ⁻¹	152	1.4 × 10 ⁻¹
 1-ethyl-1-thionia-cyclohexane iodide	85:15	1.2 × 10 ⁻¹	110	
 4-methyl-1, 4-oxathianium iodide	85:15	1.3	132	7.2 × 10 ⁻²
CH ₃ (CH ₂) ₅ S (CH ₃) ₂ I Dimethyl <i>n</i> -hexyl sulphonium iodide	85:15	2.1 × 10 ⁻²	74	
(CH ₃) ₃ S Br trimethyl sulphonium bromide	85:15	1.3 × 10 ⁻²	82	
(CH ₃) ₂ S(CH ₂) ₄ S(CH ₃) ₂ 2I 1,4-bisdimethyl sulphonium butane	85:15	2.3 × 10 ⁻⁵	147	
 1,2-bisthionium methane	85:15	4 × 10 ⁻⁴	144	

*Electrolytes found by Takahashi to be electronically conducting.

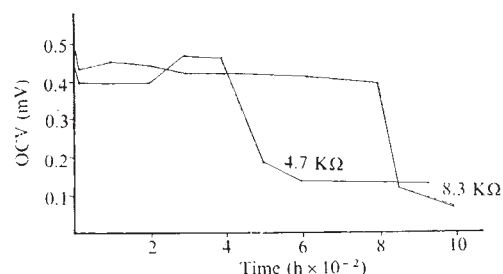


Fig. 2 Battery discharge curves for the perylene-I₂ cathode system under resistive loads of 4.7kΩ and 8.3kΩ.

The battery test cells were in the form of three layer disks; a copper anode, an 84 mole % CuI: 16 mole % 4-methyl-1, 4-oxathianium iodide electrolyte, and a cathode. The anode was formed by compressing a mixture of fine, ultrapure copper powder with a small amount of electrolyte. The electrolyte layer was added to the compressed anode and pressed further. Finally one of a variety of cathode materials was added and compaction was completed. The three layer disk was enclosed between gold-plated contact plates and encapsulated in epoxide resin, which was separated from the test cell by an insulating paper collar.

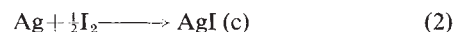
The cathode materials used were: the triiodide of the parent electrolyte; perylene-I₂; and graphite-I₂. The open cell voltages (OCV) are summarised in Table 2, which shows that the thermodynamically expected voltage was not obtained.

For the graphite-I₂ system, this could be because the partial pressure of iodine is too high; for the triiodide it is possible that compatibility⁶ or interface problems are involved.

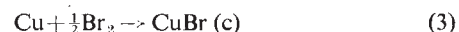
Figure 2 gives discharge curves for the perylene-I₂ cathode system under different resistive loads. Neither the OCV nor the voltage under load corresponds to the value of 0.72V expected for the reaction:



This is in contrast to the behaviour found for battery systems based on silver iodide using perylene-I₂ cathodes, where after making allowance for the iodine partial pressure, the voltage obtained accords with a cell reaction:



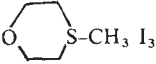
The failure of perylene halogen cathodes to act as a source of reducible halogen has been observed in other cuprous-ion solid-state battery systems. Lazzari and Pace¹¹ studied a battery involving a CuBr:N-Ndimethyltriethylenediamine dibromide electrolyte and a perylene-Br₂ cathode. They found the OCV was initially high, but rapidly dropped to 0.35V. This differed considerably from the thermodynamically expected voltage for the reaction:



Takahashi and Yamamoto¹² found a similar rapid decrease in OCV to 0.35V, for a battery involving a CuBr:C₆H₁₂N₄CH₃Br electrolyte and a perylene-Br₂ cathode. They showed, however, that it was possible to form a battery that gave a voltage corresponding to the cell reaction shown in equation (3), if the perylene-Br₂ cathode was replaced by a graphite-Br₂ cathode.

Such results show that: (1) the perylene-I₂ cathode behaves as an iodine cathode when used in a silver ion solid-state battery, but neither perylene-I₂ nor perylene-Br₂ behave in the expected way when used in cuprous halide systems. (2) It is possible to obtain a voltage corresponding to the formation of CuBr, as shown by the results of the graphite-Br₂ system. (3)

Table 2 Results of OCVs for different cathode materials

Cathode material	Predicted reaction product	Calculated OCV (V)	Experimental OCV (V)
I ₂ -perylene	CuI	0.72	0.42
I ₂ -graphite	CuI	0.72	0.02
 Triiodide derivative of sulphonium	CuI	0.72	0.05–0.3*

*Unstable.

The 'anomalous' voltage obtained in cuprous-perylene systems is not achieved immediately, which implies a possibility of some initial chemical reaction, which precedes the electrochemical reaction, taking place within the cathode. Alternatively the delay could arise because of electrolyte-electrode interface problems; a similar initial period of instability was observed in our conductivity cells.

The unexpected results obtained for the systems involving perylene cathodes could perhaps be caused by an oxidation of cuprous to cupric ion (the corresponding oxidation not being feasible for silver). Alternatively, the product of the cell reaction could be a complex involving copper, perylene and a halide. A third possibility, that the reaction product involved solely copper and perylene, was eliminated by forming a cell consisting of the normal anode and electrolyte in conjunction with a cathode solely consisting of perylene. No OCV was observed.

As perylene-I₂ systems failed to give the anticipated OCV, unstoichiometric Cu₂S was tried as an alternative cathode material. This cathode has been successfully used by Takahashi and Yamamoto¹² in CuBr-tetra-alkylammonium systems, for which the thermodynamically expected voltages were obtained. We found, however, that only a negligible voltage was produced by a battery comprising the usual anode and electrolyte with a cathode containing Cu₂S, S, C and electrolyte.

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Least radiogenic terrestrial lead from Isua, West Greenland

FIELD relationships and geochronological data have shown that the metamorphosed supracrustal rocks of the Isua area are the oldest known components of the Archaean complex of West Greenland^{1–4}. Rb-Sr, U-Pb and Pb/Pb ages in the range ~ 3,700–3,800 Myr have been reported from various units within the Isua supracrustals^{5–9}. One of us (P.W.U.A.) has recently discovered occurrences of sulphide mineralisation, including galena, within the Isua supracrustals. Here we describe these occurrences and report Pb isotopic analyses for several galenas, one of which is uniquely unradiogenic for a terrestrial rock in both ²⁰⁶Pb/²⁰⁴Pb and ²⁰⁷Pb/²⁰⁴Pb ratios. Until now the least radiogenic ore leads came from the Barberton Mountain Land of southern Africa (see refs 10, 11) and from the Pilbara Goldfield region of Western Australia¹². The least radiogenic rock leads have been reported from the ~ 3,600–3,700-Myr-old Amitsoq gneisses of West Greenland^{13–15}.

The Isua supracrustals contain major sequences of amphibolites, probably representing metamorphosed basaltic lavas and tuffs, which have undergone several phases of deformation^{16,17}. Within the amphibolites there are extensive zones mineralised with pyrrhotite and chalcopyrite, locally with minor galena and sphalerite.

Three galena-bearing horizons within the amphibolites were sampled. At locality A (Fig. 1) two parallel horizons, several metres apart and traceable for 60 m along strike, are 0.5–1-m thick and consist of dense, dark-green to black amphibolite with minor galena (samples 2946 and 2954) and traces of sphalerite and Ni-Sb sulphosalts. The fine-grained amphibolite is composed mainly of hornblende, plagioclase and quartz, with minor biotite and epidote, and occasional thin, carbonate-bearing bands. The amphibolite is strongly deformed, resulting in disruption of hornblende and plagioclase grains. The sulphides occur as anhedral grains disseminated throughout the rock, with a slight tendency to form thin, parallel layers. The grain size averages ~ 0.5 mm. Sulphides also occur within thin, discordant veinlets, rarely exceeding several millimetres in width and several centimetres in length.

The third galena-bearing amphibolite (locality B, Fig. 1) is medium-grained and rather rich in quartz, with up to 10% by volume of sulphides. The principle sulphides are pyrrhotite, chalcopyrite, sphalerite and galena, with grain size averaging ~ 0.2 mm, and occurring in thin, parallel layers. This amphibolite is cut by a ~ 5-m wide shear zone

Table 1 Pb isotope data

Sample no.	²⁰⁶ Pb/ ²⁰⁴ Pb	²⁰⁷ Pb/ ²⁰⁴ Pb	²⁰⁸ Pb/ ²⁰⁴ Pb
NBS-982			
recommended value	36.7390	17.1597	36.7449
Run 1	36.6087 ±0.0096	17.0759 ±0.0027	36.5078 ±0.0088
Run 2	36.6243 ±0.0199	17.0829 ±0.0079	36.5115 ±0.0212
Average fractionation	–0.3335%	–0.4680%	–0.6403%
2946	11.434	13.345	31.424
2954	11.428	13.335	31.383
2926	11.314	13.158	31.301
2926	11.326	13.175	31.335
2966	11.146	13.025	31.148
2966*	11.151	13.037	31.179
2966*	11.164	13.052	31.216

Replicate analyses represent separate sample dissolutions.
*Analysed without purification; all other samples purified by electro-deposition technique¹⁸.

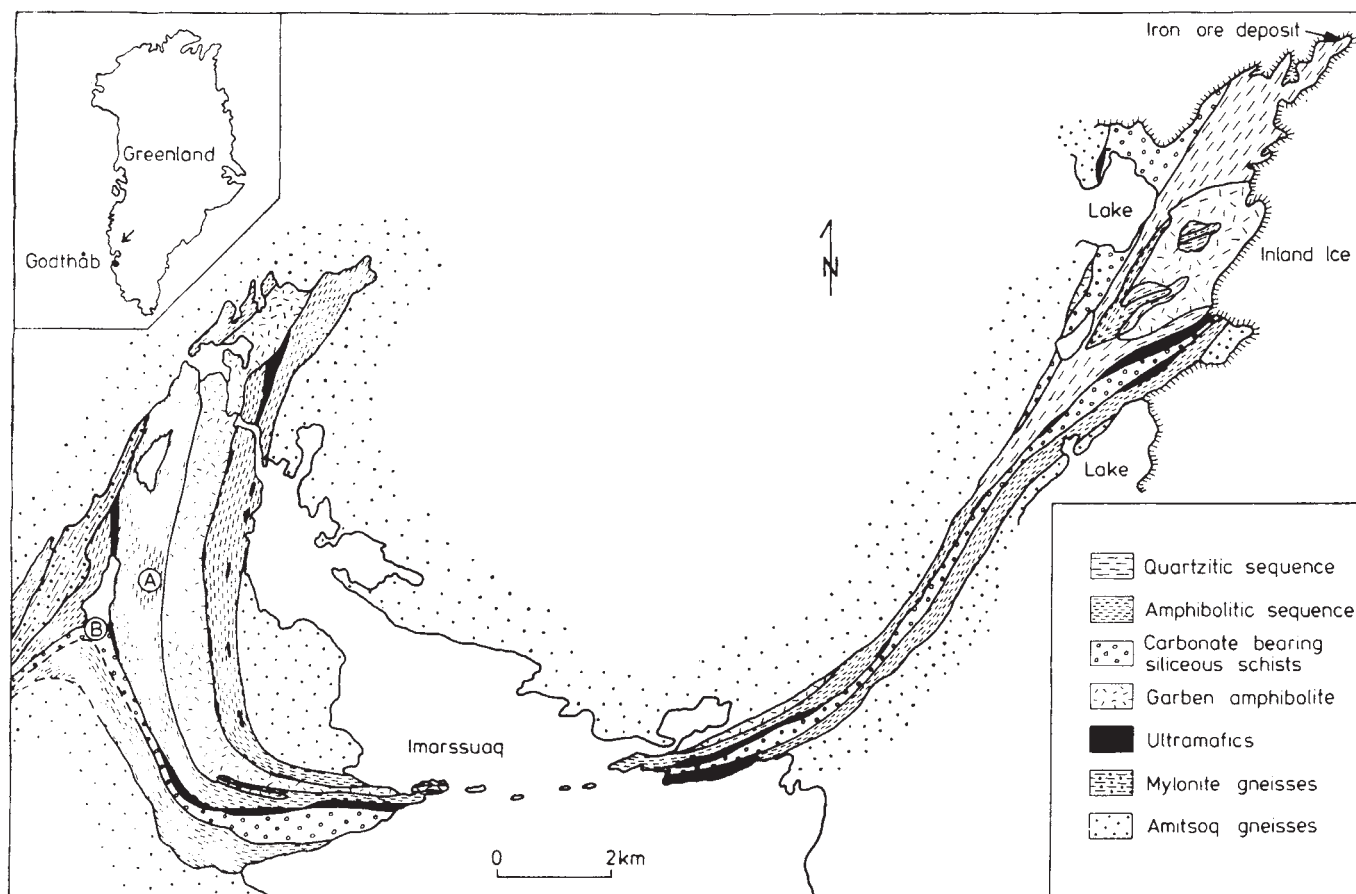


Fig. 1 Simplified geological sketch map of Isua after Allaart^{3,4}, showing specimen localities A (samples 2946 and 2954) and B (samples 2926 and 2966).

characterised by bright green, closely jointed, fuchsite-bearing quartzite. Several joints contain galena with minor sphalerite, with grain size up to ~ 2 mm (sample 2966). The shear zone further contains several discordant quartz veins, up to 0.5 m wide, carrying traces of chalcopryrite, pyrrhotite and galena (sample 2926).

Hand-picked grains of galena were dissolved in concentrated HNO_3 . Some samples were analysed without further purification, and others were prepared by the double electrodeposition method of Arden and Gale¹⁸. Pb samples were loaded on single Re filaments with H_3PO_4 and silica gel, and isotopically analysed with a 30-cm radius, 90° sector solid-source mass spectrometer. Within-run precision on all Pb isotopic ratios is better than 0.06%. Two runs of NBS 982 Pb isotope standard are reported in Table 1, from which we apply a mass fractionation correction factor of -0.16% per atomic mass unit. The uncertainty on individual Pb isotopes ratios is $\sim 0.1\%$.

Pb isotopic analyses of the galenas are presented in Table 1 and plotted in a $^{207}\text{Pb}/^{204}\text{Pb}$ – $^{206}\text{Pb}/^{204}\text{Pb}$ isotopic evolution diagram (Fig. 2). The least radiogenic Amitsoq gneiss Pb isotopic compositions and the initial Pb isotopic compositions proposed for the Amitsoq gneisses (Gancarz and Wasserburg¹⁹ and P.N.T. and S.M., unpublished) are also plotted in Fig. 2.

Sample 2966 has the most primitive $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{207}\text{Pb}/^{204}\text{Pb}$ yet reported for any terrestrial rock or ore Pb. Not even the proposed initial Pb isotopic compositions of the Amitsoq gneisses are quite as primitive. The $^{206}\text{Pb}/^{204}\text{Pb}$ ratio of sample 2966 is also very primitive, although Baadsgaard *et al.*¹⁴ have reported slightly lower values from several Amitsoq gneiss feldspars. The other Isua galenas also have very unradiogenic Pb isotopic compositions, although only the $^{206}\text{Pb}/^{204}\text{Pb}$ of sample 2926 is

significantly less radiogenic than all other published values for terrestrial Pb.

The observed differences in the Pb isotopic compositions of the Isua galenas (Table 1, Fig. 2) can be variously interpreted. A simple single-stage model for each ore Pb sample yields the model parameters shown in Table 2. On this interpretation each galena contains Pb extracted at slightly different times from sources with slightly different $^{238}\text{U}/^{204}\text{Pb}$ (μ_1) values. In geological terms it is probably more realistic to regard each Pb as mobilised and isolated from different $\sim 3,700$ – $3,800$ -Myr-old Isua supracrustals with individually variable U/Pb ratios during regional heating caused by emplacement of the magmatic parents of the Amitsoq gneisses some 3,600–3,650 Myr ago, although not all the mineralisations need be absolutely contemporaneous. It is not possible at this stage to set narrower constraints with so few available samples. However, the data definitely show that the galenas form part of the oldest sulphide mineralisation yet discovered. An extrapolation of the best-fit regression line through the

Table 2 Model ages of Pb ores and model geochemical parameters of source region

Sample no.	Model age (Myr)	$^{238}\text{U}/^{204}\text{Pb}$ (μ_1)	$^{232}\text{Th}/^{204}\text{Pb}$
2946	3640	7.77	34.45
2954	3640	7.74	33.68
2926	3620	7.25	32.07
2966	3740	7.52	33.76

Constants used in model calculations^{19–21}: age of Earth, 4570 Myr; uranium decay constants ^{238}U , $0.155125 \times 10^{-9}\text{yr}^{-1}$ and ^{235}U , $0.98485 \times 10^{-9}\text{yr}^{-1}$; initial meteoritic Pb isotope composition $^{206}\text{Pb}/^{204}\text{Pb} = 9.307$, $^{207}\text{Pb}/^{204}\text{Pb} = 10.294$.

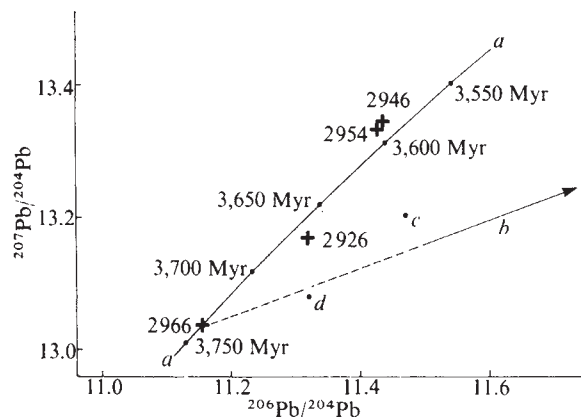


Fig. 2 Pb isotopic data for Isua galeas (Table 1) plotted in relation to single stage growth curve (line *a*) with primary $^{238}\text{U}/^{204}\text{Pb}$ (μ_1) = 7.52. For model parameters used, see Table 2. The μ_1 has been chosen so that sample 2966 lies on the growth curve. Continuous line *b* is the present-day range of least radiogenic Amitsoq gneiss rock leads (ref. 13, and unpublished data). Points *c* and *d* are respective initial Pb isotopic ratios for Amitsoq gneisses proposed by Gancarz and Wasserburg¹⁵ and by P.N.T. and S.M. (unpublished).

Amitsoq gneiss rock leads of the Godthaab area, some 100 km south-west of Isua, passes through sample 2966 (Fig. 2). The slope of the regression line corresponds to a Pb/Pb age of $3,770 \pm 160$ Myr. It could be argued that sample 2966 represents the initial Pb isotopic composition of the Amitsoq gneisses, but we do not favour this hypothesis because initial Pb isotopic ratios proposed for the Amitsoq gneisses by Gancarz and Wasserburg¹⁵ and by us (unpublished), based on direct measurements of U and Pb contents, are not as unradiogenic as sample 2966 (Fig. 2). Furthermore, field evidence clearly suggests derivation of Pb mineralisation from the Isua supracrustals and not from the Amitsoq gneisses.

Because of the variability in isotopic composition of these ancient, unradiogenic Pb ores, as well as the complexities in mode of field occurrence and geological history, we do not believe that the Isua galeas necessarily constitute appropriate data points for constraining models for terrestrial Pb isotopic evolution or for estimating the age of the Earth. It is quite feasible that even less radiogenic ore Pb than sample 2966 will be found at Isua.

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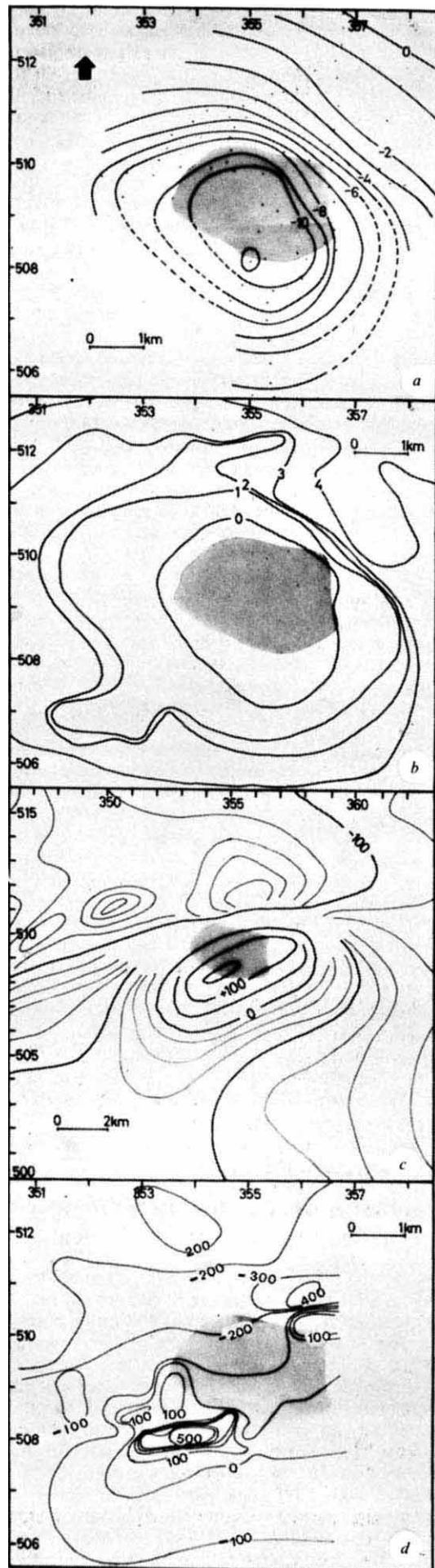
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Geophysical constraints on structure and emplacement of Shap granite

To understand Caledonian granite genesis in Britain it is essential that studies into the two-dimensional surface features of intrusions continue into the third dimension using various geophysical techniques. The characteristics of continental crust stabilised in the British Caledonian have been discussed elsewhere¹ and models for their evolution suggest a derivation direct from the mantle or by partial fusion of the oceanic crust. The Shap granite is no exception: geochronologically, geochemically and palaeomagnetically² it belongs to the Devonian group of Lake District granites, including the Eskdale, Skiddaw and Sub-Pennine Weardale intrusions. This suite yields the low initial $\text{Sr}^{87}/\text{Sr}^{86}$ ratios (~ 0.706) (P. J. O'Connor, G. C. B. and M. D. Max, in preparation) which are characteristic of mantle fusion followed by limited crustal scavenging. The Shap intrusion occurs in the eastern Lake District and outcrops as an irregular oval measuring 2.8 km east-west and 2 km north-south. It is a porphyritic adamellite³ dated 393 ± 11 Myr (ref. 4) and occurs at the junction between Ordovician Borrowdale volcanics and Silurian Brathay flags. The granite possesses a broad metasomatic aureole containing grossular and epidote. There has been debate concerning the subsurface shape of the intrusion, which may be lopolith⁵ or cone-shaped with depth. The gravity survey of the Lake District⁶ strongly suggests the latter, but modelling was based on only 14 stations along a single linear traverse of the granite. A stock-like body, extending to 8 km depth, gave a satisfactory model at that time. Here we report the combined observations of ground-based gravity and magnetic field surveys which apply to the structure of the intrusion and its aureole rocks.

Figure 1a is a bouguer anomaly map of the intrusion prepared by levelling and measuring 66 gravity stations within and around the granite outcrop. The negative anomaly of 11 mgal is caused by the mass deficiency of the granite (density = $2.665 \times 10^3 \text{ kg m}^{-3}$) compared with the country rocks (density = $2.789 \times 10^3 \text{ kg m}^{-3}$) and the displacement of the anomaly to the south of the outcrop is due either to a less dense granite (see ref. 6), or an off-setting of the three-dimensional granite body to the south of the outcrop. The bouguer gradient is relatively shallow except in the north-east, where the steepest gradient (4 mgal km^{-1}) occurs indicating a steep-sided, possibly fault-controlled boundary with Borrowdale volcanics at depth. The concentric anomaly pattern of Fig. 1a supports the general conclusion that we have a steep-sided, cone-shaped intrusion with depth. To refine this conclusion the anomaly for a three-dimensional model of the intrusion was computed⁷ and is shown in Fig. 1b. The surface contour fits the granite outcrop pattern to a good approximation. It is clear that the intrusion slopes down to 4 km depth, the apparent



limit of this survey area, at angles varying from 75° (north-east margin) to 20° (south and west margin), although a gradual steepening can be inferred towards the boundary of the survey area. A concentration of the granite mass occurs to the south and south-west of the main outcrop and coincides with the anomaly centre (Fig. 1a). These conclusions are clear and decisive in terms of model shape but not unexpected for a low-density granite stock intruding into intermediate-composition country rocks. More surprising is the aeromagnetic anomaly pattern extracted from the IGS map series⁸ and shown in Fig. 1c. The anomaly is simple and comprises a closed positive (+150 nT) to the south with a corresponding negative (−140 nT) to the north of the outcrop. The magnetic gradient is strongest across the granite outcrop which suggests a source for the anomaly within the intrusion, and a predominant induced component of magnetisation is implied by the present magnetic inclination at Shap. Indeed, measurements of susceptibility and remanence from granite samples (Table 1) record the anticipated predominantly induced magnetism, but an attempt to model this field using these values and the shape deduced by gravity methods (Fig. 1b) gave an amplitude of only 50 nT and not the 300 nT recorded aeromagnetically. A possible solution would be a vertically inhomogeneous intrusion possibly becoming more iron-rich and presumably basic, with depth. Any consequent reduction in density contrast between the intrusion and its envelope would then require a considerably decreased dip of the intrusion walls with depth in order to increase its volume. Adjustments of the required magnitude seemed untenable, so a ground-based survey was carried out in an attempt to distinguish in more detail the exact source of the magnetic anomaly.

About 1,000 measurements from field studies by Liverpool and Cambridge geophysicists are synthesised in Fig. 1d—the ground-based magnetic anomaly map—where limited optical smoothing has been adopted. At ground level the amplitude of the magnetic anomaly becomes 700 nT but, more significantly, two centres of strong magnetisation are resolved. These are located on the north-east and south-west intrusion margins, leaving a relatively small magnetic gradient (130 nT km^{-1}) across the granite outcrop compared with anomalous regions (approaching 500 nT km^{-1}). Significantly, our earlier conclusion, based on the aeromagnetic map, that the intrusion is the main anomaly source, is now proved invalid and this is a warning against the use of aeromagnetic data in geological terms. A country rock source, marginal to the intrusion, is more appropriate, and the earlier misinterpretation is attributed to the smoothing effect of contouring aerial data and using widely spaced flight lines (usually 2 km separation). These factors are responsible for the essential contrasts between Fig. 1c and 1d.

The country rocks in both anomalous regions are andesitic ashes and rhyolites of the Borrowdale series, just north of the Ordovician–Silurian boundary. Suspecting that the magnetisation is a contact metamorphic effect, we collected a suite of samples from the south-west margin at various distances from the granite contact. Exposure problems prevented this treatment for the north-east anomaly, but near contact sampling was possible on the magnetically quiet northern margin. Laboratory magnetic analyses in

Fig. 1 a, Bouguer anomaly map of the Shap granite; contours at 1 mgal intervals; b, contour map of the intrusion model computed from the Bouguer Anomaly (see text); contours show the depth to the granite intrusion and are 1 km apart; c, aeromagnetic contours in the Shap granite area; contours at 50 nT intervals; d, ground-based magnetic map of the Shap granite; contours at 100 nT intervals. Shaded area indicates the surface outcrop of the granite with respect to national grid references.

Table 1 Comparison between mean induced and remanent magnetic components for Shap granite and Borrowdale volcanic samples

	Induced magnetisation ($\times 10^{-3}$ e.m.u. g $^{-1}$)	Remanent magnetisation ($\times 10^{-5}$ e.m.u. g $^{-1}$)
Shap granite	18.5	0.9
Borrowdale volcanics (1 m from contact)	114.7	28.6
Borrowdale volcanics (20 m from contact)	25.4	1.2
Borrowdale volcanics (magnetically quiet areas)	4.0	2.4

Table 1 show that the induced magnetisation 1 m from the contact is extremely high (114.7×10^3 e.m.u. g $^{-1}$) and decreases by an order of magnitude within 20 m from the intrusion. Samples from the quiet areas have induced magnetisation a further order of magnitude lower. Samples close to the contact also have fairly high remanent magnetisation (Table 1), but this fades away rapidly with distance and becomes somewhat erratic. In any case, the remanent magnetisation contributes only a small proportion of the total.

Petrologic examination of the magnetic samples reveals the presence of large magnetic grains (up to 500 μ m), which are likely to be the chief carriers of magnetisation (a measured Curie Point was 580 °C) rimmed by haloes of potash feldspar. Elsewhere, in thin sections from the magnetic region, the ubiquitous presence of biotite, hydrothermally replacing pyroxene⁹, and now in reaction relationship with the magnetite-K feldspar assemblage, suggests the reaction:



In thin sections this reaction proceeds more strongly to the right on approaching the granite contact which indicates a dehydration process during contact metamorphism. This also occurs on a limited scale in xenolithic fragments from the Shap granite quarry. Andesite thin sections from the magnetically quiet region contain little biotite or magnetite; pyroxene is the chief ferromagnesian constituent.

We conclude that the strong positive magnetic anomaly associated with the south-west corner of the Shap granite is due to a combination of remanent and predominantly induced magnetic components within contact metamorphic magnetite crystals. Models of the body creating the south-west anomaly were computed¹⁰ along three north-south traverses at increasing distance from the granite. Obviously, many models would provide a reasonable fit, but we favour a polygonal body 0.75 km high, 0.5 km wide at the top increasing to 1 km at the bottom. The top of this body, a magnetised skin of Borrowdale volcanics, dips away from the granite outcrop to the west as seen in the three traverses at 20°; this correlates well with the model produced from the gravity data which in this area has a dip of 18° (see Fig. 1b). The anomaly to the north-east of the intrusion is incomplete in this survey and has not been studied petrologically, but again it seems to have predominantly induced magnetisation which may be due to similar metamorphic effects on the same units of the Borrowdale volcanics, for the regional strike direction is north-east-south-west across the granite. In the Eastern Lake District values for the regional dip of the Borrowdale volcanics range over 20° and 60° between south and south-east^{11,12}. On each of the three north-south traverses the magnetic model dip component to the south was 40° which correlates well with these direct field estimates. This correlation supports the notion of a contact metamorphic origin for the two magnetic anomalies associated with the Shap granite.

Our studies of the Shap granite show it to be a steep-sided cone-shaped intrusion whose central axis is displaced about 1/2 km south of the outcrop centre. We suspect it to be fairly homogeneous with depth and from independent data¹⁻² to be derived as a potash tonalite-adamellite at mantle depths, possibly within or above subducted ocean crust¹³, followed by limited fractionation and crustal assimilation on ascent. These features are reflected in the low initial Sr⁸⁷/Sr⁸⁶ ratio for the Shap granite (0.70767) recently determined by Gale and Wadge (personal communication). The intrusion is emplaced into intermediate volcanics some of which are rich in biotite, some of which in turn dehydrated to magnetite during contact metamorphism. This magnetite produces an unusually strong magnetic anomaly for granite terrain. Also the use of aeromagnetically smoothed data to unravel detailed geological problems can lead to erroneous deductions concerning anomaly source, and we urge caution in future small scale geophysical interpretations of this nature.

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Vertical zonations of marsh foraminifera as accurate indicators of former sea-levels

MANY attempts to reconstruct the pattern of apparent sea-level rise that took place during the Holocene have used salt-marsh deposits as indicators of former sea level^{1,2}. Contemporary mature salt marshes occupy a vertical range covering the upper half of the tidal range, from mean sea level to higher high water^{3,4}, and geologists have arbitrarily equated ancient marsh deposits with various fixed elevations within this range. This method, disregarding any other source of inaccuracy, can introduce an error of 1-5 m depending on the tidal range. Apparent sea level movements during the late Holocene (2,000-3,000 yr BP) have been of the order of 1-2 m; hence such a method introduces errors larger than the movements being measured. Even less adequate sea-level estimates are derived from other commonly used indicators such as raised beaches, oyster beds, and submerged or

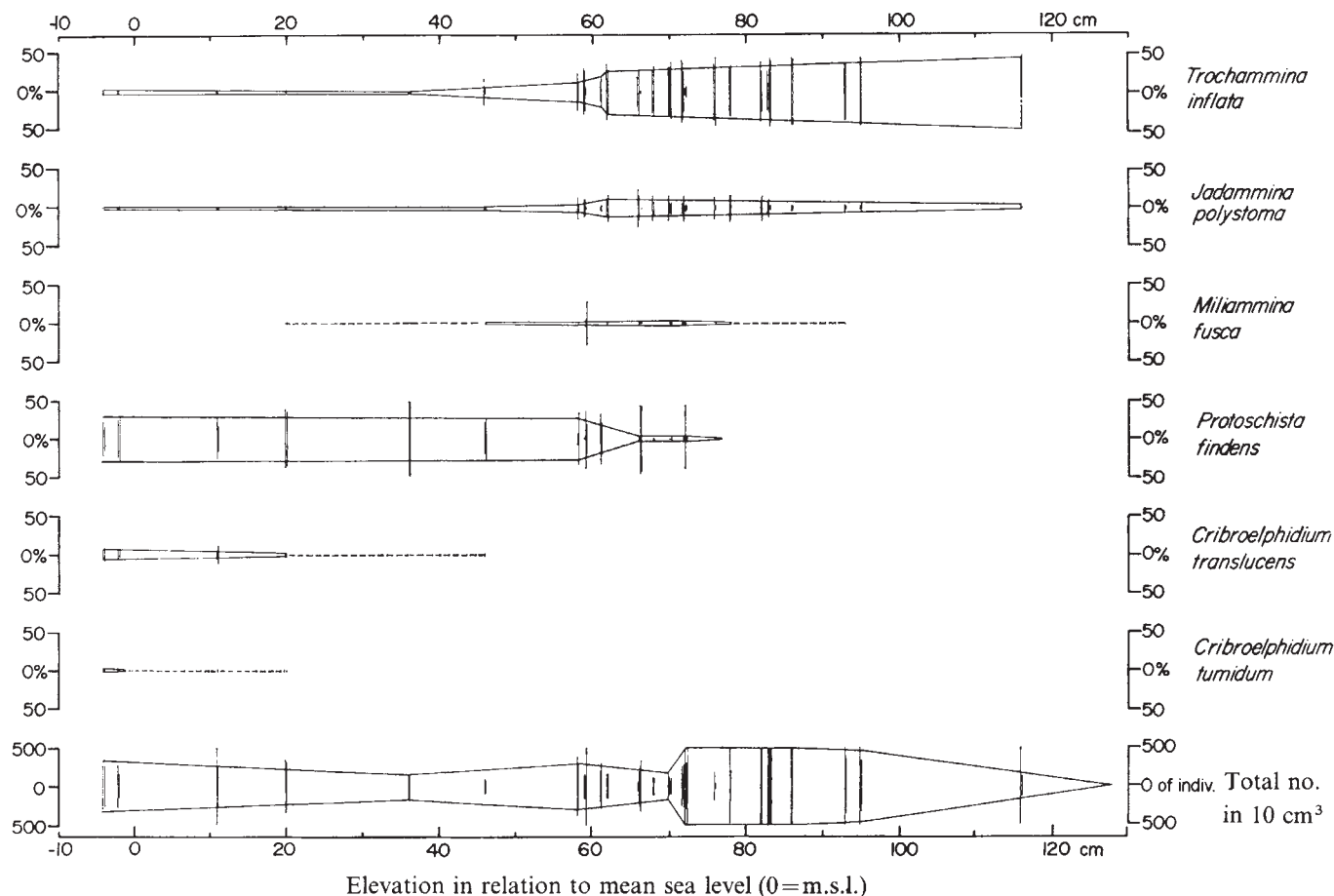


Fig. 1 Marsh foraminiferal distribution along a vertical scale in Tiajuana Lagoon, California. Vertical bars represent percentages of total populations for each species at different elevations except on lowest bars where the vertical lines indicate absolute total numbers. Horizontal connecting lines indicate averaging, hence vertical lines ending either outside or inside these lines are intrazonal rather than interzonal variations. The total numbers of some populations are as high as 10,000 but the scale is limited to 1,000 as variations between 1,000 and 10,000 seem to be random and have little significance. The upper four species displayed are agglutinated while the bottom two are calcareous forms.

emerged terraces and deltas. We describe here how marsh foraminifera can be used to delimit small-scale vertical zones along modern marsh surfaces generally corresponding to the floral zones in vertical range. This relationship was first established in the marshes of southern California⁵, and has recently been shown to be valid in other areas⁶.

A marsh foraminiferal zonation is a significantly more accurate indicator of sea level than undifferentiated marsh deposits, because well defined zones subdividing the marsh increase the vertical resolution of the deposits. Marsh plants also form vertical zones³ but their poor preservation in subsurface deposits renders them less suitable than marsh foraminifera with agglutinated tests, which resist solution in the low pH of the marsh sediments and are well preserved in subsurface sediments. Additionally, the latter occur in high numbers (100–200 cm⁻³), thus providing a good statistical base. The relationship between vegetation zones and foraminiferal assemblages in modern salt marshes has been suggested^{7–9} but these earlier studies were designed to demonstrate broad regional relationships and did not define the fine scale correlations between foraminiferal assemblages, plant zones, and elevation that are required for accurate spatial measurements of sea level.

To detect the small-scale zonations within a marsh, closely spaced samples with accurate vertical and horizontal control were required. This we achieved by careful use of a transit along transect lines with elevations being measured to the nearest cm in relation to nearby benchmarks.

Zonal assemblages were established on the basis of total populations (living + dead) rather than on living populations alone. This is more reliable: total populations integrate all seasonal variations thus eliminating the risk of over-emphasising individual species due to seasonal fluctuations¹⁰. Surface samples were collected in replicates of 10 cm³ (10 cm² × 1 cm) each, making them comparable to those of previous workers.

Two examples, one from Tiajuana Lagoon, California (32°N, 117°W, 8°W) and one from Chezzetcook Inlet, Nova Scotia (44°N, 63°W, 15°W), are shown here for comparison. Nova Scotian and southern Californian marshes were chosen because they were a maximum contrast between a temperate region (humid, cool) and an arid, warm climatic setting.

Two distinct faunal zones, with the lowest one divided into two subzones, were observed in Tiajuana Lagoon (Fig. 1): (1) a lower faunal zone at –5 to 80 cm above mean sea level (a.m.s.l.) with two subzones (–5 to 65 cm a.m.s.l. and 66 to 80 cm a.m.s.l.) and (2) a higher faunal zone at 80 to 120 cm a.m.s.l. The upper limit of the tidal influence (higher high water=HHW, 125 cm a.m.s.l.) is clearly distinguished by a marked decrease in the total foraminiferal population between 115 and 125 cm a.m.s.l.

In Chezzetcook Inlet two distinct faunal zones could also be recognised and both are divided into subzones (Fig. 2). A lower faunal zone occurs from –25 to 79 cm a.m.s.l. with two subzones (–25 to 68 cm a.m.s.l. and 69 and 79 cm

a.m.s.l.). An upper faunal zone occurs from 79 to 101 cm a.m.s.l. with two subzones (79 to 94 cm a.m.s.l. and 95 to 101 cm a.m.s.l.). As in California the foraminifera show a marked decrease in number at HHW (101 cm a.m.s.l.).

Floral zones were determined at the same time as the foraminiferal zones and these are compared in Fig. 3. Although no plant species and few foraminiferal species are common to both areas, the floral and faunal groups assemble into similar elevational patterns in both California and Nova Scotia. The marked decrease in foraminiferal numbers towards the upper limit of the tidal range (HHW) may seem a trivial observation. However, the phenomenon is not self-evident on closer examination of the area above HHW. Conditions in surface sediments above HHW might be considered favourable for support of large foraminiferal populations, especially in humid areas such as Nova Scotia where moisture is supplied both by freshwater runoff and seawater raised by capillarity, creating a mildly brackish environment. Therefore, the absence of foraminifera above HHW is significant, indicating that marsh forms require some tidal activity for survival and that it is highly unlikely that they would be found in supra-tidal bogs or other freshwater deposits resembling marsh sediments.

The data summarised in Fig. 3 suggest a strong correlation between elevation above mean sea level and marsh foraminiferal zones. The marsh foraminiferal assemblages are well preserved and easily detected in subsurface marsh deposits. Thus marsh foraminifera can be used for accurate (± 5 cm if HHW can be located) positioning of former sea levels in a marsh sequence. Although this precision has only been proved in areas where accurate measurements have been carried out such as California and Nova Scotia, less

detailed data from other areas⁷⁻⁹ suggest that similar accuracy could be obtained in any marsh.

Figure 3 shows that certain zones yield higher accuracy than others (the larger the vertical range, the lower the accuracy). The level that can be most accurately located is HHW which is particularly favourable for several reasons^{2,6,11}.

In some places the HHW data are not preserved, possibly never having formed because of low sedimentation rates in the higher areas of the marsh⁴. In these situations the lower part of the faunal zone I is usually present. Although the use of these deposits allows less accurate measurements than the HHW level, the accuracy is still significantly higher than with any previous method (± 15 cm). More important, it is now possible to assess the accuracy of the various marsh deposits as sea level indicators, rather than having to assign an arbitrary value to all ancient marsh deposits. Moreover, the mere presence of salt marsh foraminifera differentiates salt marsh from freshwater peats. Where modern assemblages are used to interpret Holocene events, a large body of detailed information covering all possible changes of the assemblage related to environmental variations is necessary for optimal results. This is seldom if ever possible and recent marsh data of the type required are particularly sparse. However, because of the extremely marginal marine conditions existing in a marsh, few species of marine organisms (foraminifera) can survive in this environment and these seem to have worldwide distribution^{12,13}. Although regional differences between marsh assemblages (such as between California and Nova Scotia) probably result from a series of environmental factors, it is interesting that foraminifera characterising the upper half of the marsh

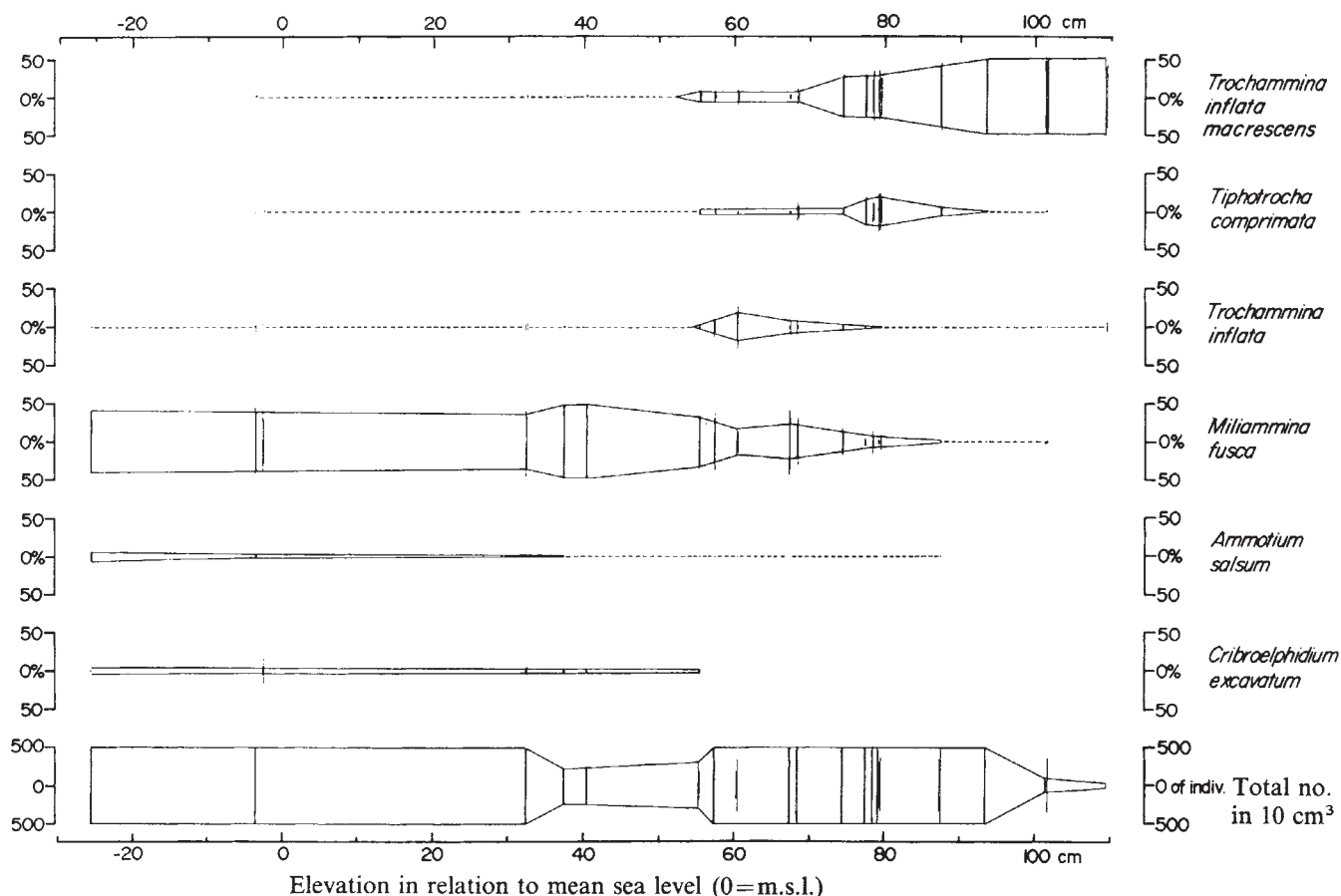


Fig. 2 Marsh foraminiferal distribution along a vertical scale in Chezzetcook Inlet, Nova Scotia. The format of this diagram is the same as for Fig. 1. All species here are agglutinated except for *Cribroelphidium excavatum* which is calcareous.

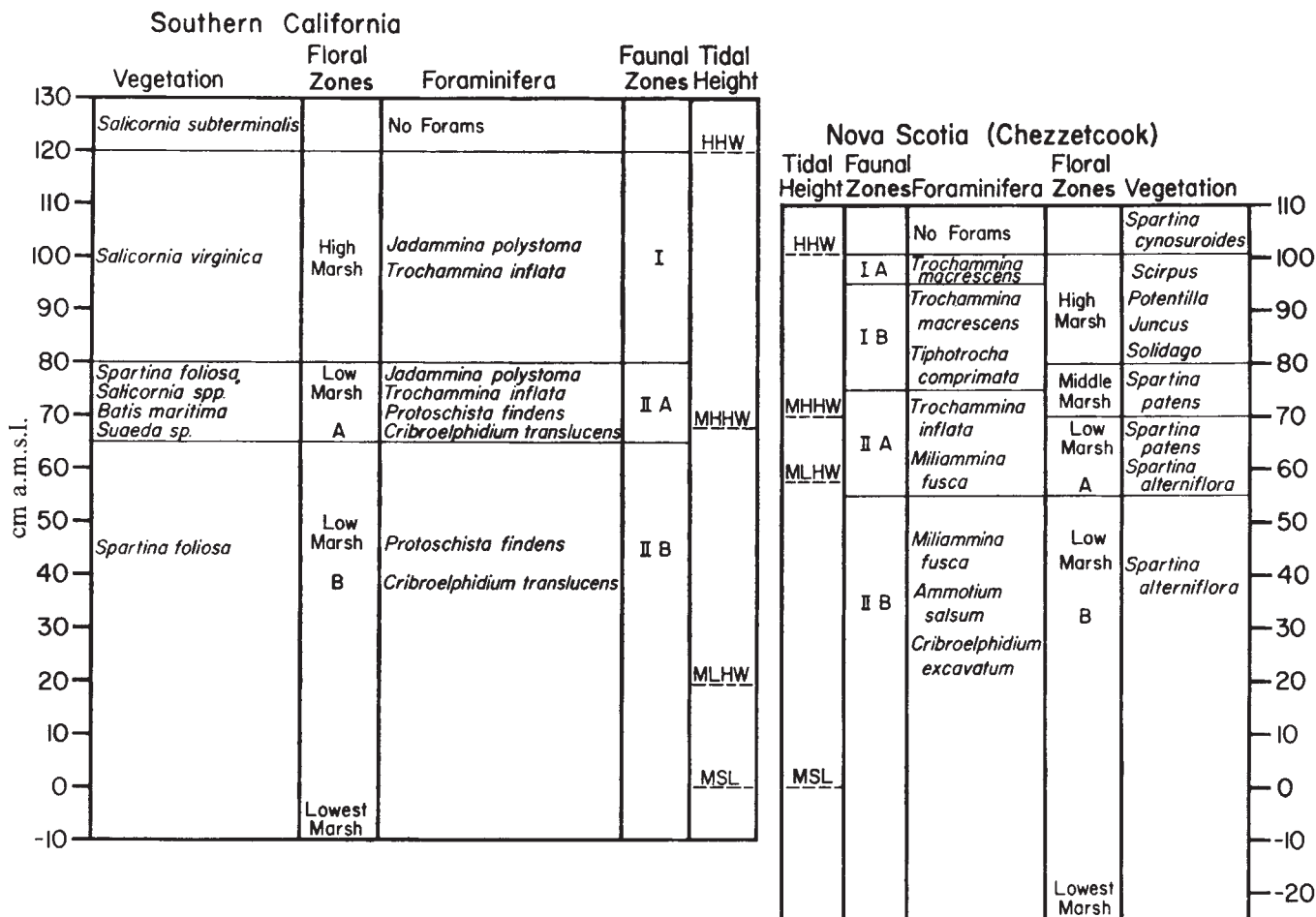


Fig. 3 Comparison chart for California and Nova Scotia marsh data. Floral zones are denoted as high marsh, middle marsh and so on, while faunal zones are denoted by numbers (I, II). Subzones are indicated as IA, IB and so on. HHW = higher high water, MHHW = mean higher high water, MLHW = mean lower high water, MSL = mean sea level.

(*Trochammina inflata*, *T. inflata macrescens*, *Tiphotrecha comprimata*, *Jadammina polystoma*) occur only in that area or not at all. Hence their distributions are limited largely by absolute elevation above mean sea level (depending on tidal range). Thus the presence of one or all of these forms as dominant species in any salt marsh deposit will normally indicate elevations in the upper quarter of the tidal range, regardless of the conditions in which the marsh deposit formed.

The accurate determination of sea level using marsh foraminifera has many potential applications. The most obvious one is the study of changing Holocene sea levels; we have already tested this in Nova Scotia⁸. The method can also be used to obtain accurate data in coastal zone planning.

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Identification of lead compounds in urban air

In the current controversy over the toxicity of environmental lead, one point still at issue is the efficiency of respiratory uptake of urban lead aerosol. The rate at which lead passes into the blood is a function of the particle size and the solubility of the deposited lead. Although data on the aerodynamic particle size distribution of urban lead aerosol are plentiful, little is known of the chemical nature, and thus solubility, of the lead and so this has hampered studies of respiratory uptake. Several analytical studies of fresh auto exhaust have been reported¹, but Ter Haar and Bayard² have shown that rapid changes occur after emission into the atmosphere. Although these workers characterised the major components of aged lead aerosol, limitations of the electron microprobe techniques which they used have been pointed out by Heide and Desborough³, and considerable doubt must attach to the structural assignments. Using two techniques to enrich the lead fraction of

Table 1 Results of XRD analyses of atmospheric particulates

Site	Site description	Date	Sampling method	Lead compounds identified
Lancaster	~ 20,000 vehicles p d ⁻¹ [Pb] ~ 1–6 µg m ⁻³	5–7/5/77	Hi-Vol	(NH ₄) ₂ SO ₄ .PbSO ₄ ; PbSO ₄ (minor)
		17–24/5/77	Andersen impactor	(NH ₄) ₂ SO ₄ .PbSO ₄ ; PbBrCl (minor)
	MMED for lead = 0.7 µm 77% of total lead is < 2.1 µm	17–19/5/77	Hi-Vol	(NH ₄) ₂ SO ₄ .PbSO ₄ ; PbSO ₄ (minor)
		24–31/5/77	Andersen impactor	(NH ₄) ₂ SO ₄ .PbSO ₄
		31/5–8/6/77	Andersen impactor	(NH ₄) ₂ SO ₄ .PbSO ₄
Preston	~ 20,000 vehicles d ⁻¹ [Pb] < 1 µg m ⁻³ MMED for lead = 0.8 µm 69% of total lead is < 2.1 µm	1–8/8/77	Andersen impactor	(NH ₄) ₂ SO ₄ .PbSO ₄ ; PbBrCl (minor)
		8–15/8/77	Andersen impactor	(NH ₄) ₂ SO ₄ .PbSO ₄
		12–19/9/77	Andersen impactor	(NH ₄) ₂ SO ₄ .PbSO ₄

urban aerosol, we have now identified the major components by X-ray powder diffraction (XRD).

First, Andersen impactors were modified to collect all particles < 2.1 µm aerodynamic diameter on a single stage. This removed larger mineral particles which would interfere in the analysis, and collected the fraction corresponding to the range of particle sizes causing the greatest health hazard by inhalation. Sampling lasted for one week to collect sufficient material for identification. Second, 48-h high volume filter samples were collected. The particles were stripped ultrasonically from the filters and those of density > 3.2 g cm⁻³ were separated by a density gradient technique⁴ before analysis.

Samples were analysed using a Philips X-ray powder diffractometer and XDC 700 camera with a monochromatic Cu K_{α1} radiation source. Diffractograms and films were analysed using the Fink search manual, a computer search program and by visual comparison with the JCPDS powder diffraction files. Verification was based on both *d*-spacing and intensity matching and the results are shown in Table 1.

The Lancaster site is next to the A6 road, and is not influenced by industrial sources of lead. The XRD results (see Table 1) indicated the persistent presence of (NH₄)₂SO₄.PbSO₄, together with lesser amounts of PbSO₄ or PbBrCl in some samples. For confirmatory evidence, samples were also collected at Preston, and again the principal lead compound was found to be (NH₄)₂SO₄.PbSO₄. The presence of this compound in the environment has not been reported previously. Its solubility is probably intermediate between the highly insoluble lead compounds such as the dioxide and carbonate, and the more soluble halides and nitrate, although other chemical species present in the lung fluid will also affect solubility.

In analysis of polluted soils and street dusts, Olson and Skogerboe⁴ found PbSO₄ to be the major lead compound. We believe that (NH₄)₂SO₄.PbSO₄ is an intermediate in the conversion of vehicle-emitted lead halides to PbSO₄, formed by reaction with atmospheric ammonia and acid sulphates. Although the results presented here refer to an atmospheric aerosol aged by up to 48 h during high volume sampling, we believe that the results provide the best available guide for selection of compounds for use in respiratory uptake studies.

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Evolution of overlapping genes

THE nucleotide sequence of the DNA of ΦX174 phage established by Sanger's group^{1,2} has revealed that this phage has two overlapping genes in which the same stretch of DNA can code for two proteins which are translated in different reading frames. That two overlapping genes occur in the same DNA genome suggests that this overlapping expression is not unique to ΦX174 but of more general significance, at least in organisms with small DNA molecules. Once the overlapping expression is established, all mutations in one gene would also alter the other gene within the region of overlap. Some of these mutations, however, may affect one protein but not the other because of codon degeneracy. In general, the evolutionary constraints on a sequence representing both proteins must be very severe. In this report we estimate the extent to which the evolution of a gene is reduced by the acquisition of overlapping expression.

All the mutations which arise on a cistron do not result in substitutions. (Here we distinguish between a mutation and a substitution. By a substitution or an acceptance of mutation, we mean a mutation which spreads through a population and replaces a previous wild-type cistron.) For a quantitative analysis of the substitution pattern of amino acids³, we have introduced the amino acid pair distance, *d*, indicating the degree of the difference of physicochemical properties of amino acids *a_i* and *a_j* by using the indices of polarity *p_i* and volume *v_i* introduced by Grantham⁴ as $d = [(\Delta p_{ij}/\sigma_p)^2 + (\Delta v_{ij}/\sigma_v)^2]^{1/2}$ where Δp_{ij} and Δv_{ij} represent polarity and volume differences caused by an amino acid substitution respectively, and σ_p and σ_v are standard deviations of Δp_{ij} and Δv_{ij} respectively. The pair distance thus defined ranges from 0.06 (Ala-Pro) to 5.13 (Gly-Trp). From an analysis of amino acid substitution patterns of homologous protein families, we have already shown that *R(d)*, the substitution frequency between amino acids relative to the random substitution frequency expected from the genetic code, depends negatively on *d* irrespective of protein families³. (The regression of *R(d)* on *d* is $R(d) = -0.80d + 2.44$, for $d < 3.5$, and the correlation coefficient is -0.966 . No substitutions are observed at $d \geq 3.5$.) We have also inferred that these accepted mutations cause no appreciable disruptions of the tertiary structure of a protein³. The mutation which causes synonymous change also must be neutral for structural constraints. Thus, some fraction of mutations which arise on a cistron results in the base substitutions in the gene and the others are eliminated from the population during evolution. Then, we introduce a coefficient *s_p(d)*, the fraction of acceptance of a mutation defined by

$$s_p(d) = \begin{cases} 1 & \text{; for } d = 0 \text{ (synonymous change)} \\ p(1 - d/d_c) & \text{; for } 0 < d < d_c \\ 0 & \text{; for } d \geq d_c \end{cases} \quad (1)$$

A mutation which causes a difference by *d* results in a substitution at the rate of *s_p(d)*. From our previous analysis³, *d_c* has the nearly constant value 3.0 for almost all the protein families. The value of *p* mostly correlates with the evolutionary rate of a gene.

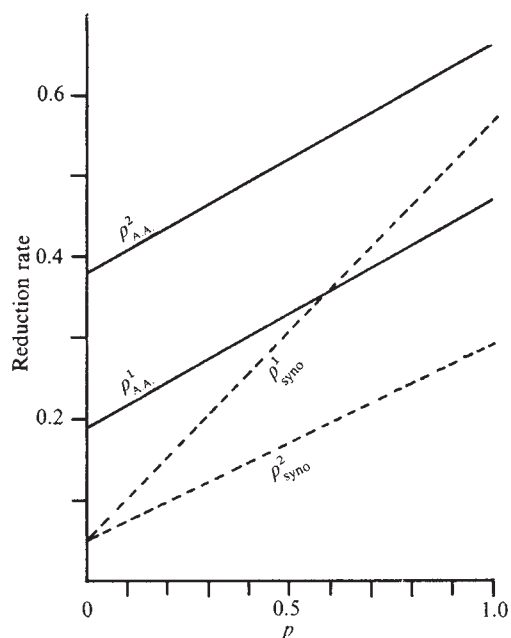


Fig. 1 The reduction of the rate of amino acid change rate $\rho_{A.A.}$ (solid lines) and that of synonymous change ρ_{syno} (dotted lines) are shown as a function of the other gene's parameter p in overlapping genes (ρ^1 for gene 1 and ρ^2 for gene 2). Ten random sequences are used in this calculation and results are averaged. Each of them consists of 300 nucleotides with equal content of the four bases.

To estimate to what extent the evolutionary rate of a nucleotide sequence representing two proteins is reduced compared with that of the nucleotide sequence coding only one protein, we assume that mutations occur uniformly on that sequence irrespective of sites and kinds of bases, and double mutations for each site are ignored. We next assume that the extent of the reduction of evolutionary rate must be proportional to the ratio of the number of substituted bases of the overlapping genes to that of non-overlapping ones. To deal with the properties of evolution of overlapping genes generally, an analysis is made using random sequences, which are 300 nucleotides long, with equal base compositions for four bases and in which no termination codons appear. The results of calculations using 10 random sequences are averaged. The direction of reading for the translation of the two proteins is the same for both genes and the first letter in gene 1 codon is the second in gene 2 and so on. The parameters p for gene 1 and gene 2 in equation 1 are p_1 and p_2 respectively.

Let $f_i(d)$ be the frequency distribution of amino acid difference

d which is caused by a mutation occurred on i th base position of a codon ($d = 0$ for synonymous change, and $d = \infty$ for a change to stop codon). As a mutation causing difference d is accepted at the rate of $s_p(d)$ according to the selection scheme of equation (1), the probability of acceptance $P_i(p)$ of a mutation occurring on the i th base position is given by

$$P_i(p) = \sum_{d=0}^{\infty} f_i(d) s_p(d) \quad (2)$$

This probability can be split into two parts, $P_i(p) = P_{i,s}(p) + P_{i,A}(p)$, that is, the probability of acceptance for a mutation causing synonymous change and that for a mutation causing amino acid change. As mutations occur with equal probability for three positions of codons, the probability of 'success' of the substitution for each mutation causing amino acid change is

$$\frac{3}{\sum_{i=1}^3 P_{i,A}(p)/3}$$

Mutations occur on a cistron independently. Thus, the number of mutations which become substitutions is represented by a binomial distribution. The expected number of substitutions causing amino acid changes, $n_{A.A.}^N$, is therefore

$$n \sum_{i=1}^3 P_{i,A}(p)/3$$

in the nonoverlapping case.

Next, we estimate the number of substituted amino acids in overlapping genes. Let us consider the evolution of a gene in which the evolutionary rate is estimated by the number of substitutions resulting in amino acid changes. Let the parameter p of the gene under consideration be p_1 and that of the other gene be

Fig. 2 Frequency distributions of the amino acid distance caused by a mutation, $f_i(d)$, and the rate of acceptance of the mutation at that distance (shaded area), $f_i(d)(1-d/d_c)$, for each base position of codons. SY, synonymous change; ST, change to stop codons. *a*, First position; *b*, second position; *c*, third position.

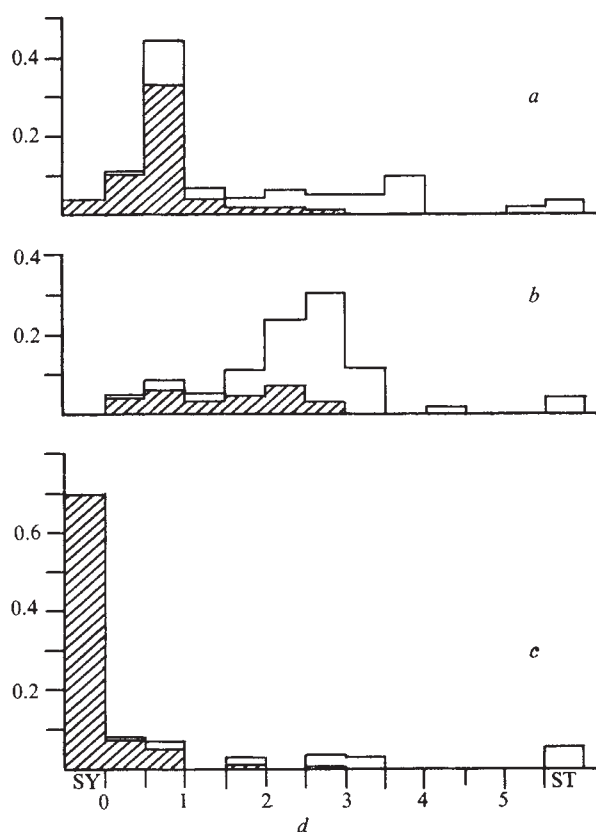


Table 1 Coefficients for random chains with equal content of the four bases and for genes in overlapping regions of $\Phi X174$

	Average of 10 random chains			$\Phi X174$ overlapping regions ($d_c = 3.0$)	
	$d_c = 2.5$	$d_c = 3.0$	$d_c = 3.5$	A-B genes	E-D genes
A	0.04 (0.03)	0.04 (0.03)	0.04 (0.03)	0.01	0.01
B	0.11 (0.12)	0.17 (0.18)	0.23 (0.24)	0.18	0.16
C	0.31 (0.33)	0.34 (0.36)	0.38 (0.39)	0.32	0.42
D	0.18 (0.16)	0.25 (0.23)	0.34 (0.32)	0.33	0.29
E	0.73 (0.73)	0.73 (0.73)	0.73 (0.73)	0.68	0.73
F	0.76 (0.76)	0.90 (0.90)	1.04 (1.04)	1.03*	0.98*

Values in parentheses are results calculated by independent approximation.

*The values for the A and B genes are 1.06 and 0.99 respectively, and for the E and D genes 0.94 and 1.01 respectively. Values shown are averages.

Table 2 Nucleotide substitution rates in translated regions

Species compared	(1) Total no. of nucleotides compared	(2) Total no. of changes	(3) Changes causing amino acid substitutions	(4) Synonymous changes	(5) f_0 , the ratio of (4) to (2)
β -globin of rabbit and human	159	18	4*	14	0.78
Histone IV of <i>S. purpuratus</i> and <i>pictus</i>	78	9	1	8	0.89
MS2 and R17	612	26	4	22	0.85
MS2 and f2	290	11	4	7	0.64
R17 and f2	322	13	3	10	0.77
Total	1,361	77	16	61	0.79

Sequence data for rabbit β -globin were taken from Browne *et al.*⁵, for human β -globin from Marotta *et al.*⁶ and Salser *et al.*⁷, for histone IV from Grunstein and Schedl⁸ and for MS2, R17 and f2 from Min Jou and Fiers⁹. For references to R17 and f2 see ref. 9.

*The number of observed changes is three, but the observed Val-Cys substitution corresponds to a two-step change. Thus, an intermediary amino acid must have appeared during evolution.

p_2 , and the relative shift of reading frame of the other gene to the gene under consideration be θ ($\theta = 1, 2$). If we assume that two nucleotide sequences, which are generated from the same stretch of DNA by different reading frames, are mutually independent (independent approximation), the same distribution $f_i(d)$ can be used for two genes. A mutation which occurs in the i th position of gene 1 and causes an amino acid change is accepted at the probability $P_{i,A}(p_1)$. This mutation, at the same time, changes the base of $i + \theta$ th position of a codon of gene 2 and is accepted at the probability $P_{i+\theta}(p_2)$. Therefore, the probability that a mutation on gene 1 is accepted by two independent selections is $P_{i,A}(p_1)P_{i+\theta}(p_2)/3$, where $P_{\theta+3n}(p) = P_\theta(p)$. If n mutations occur on gene 1, the number of substitutions $n_{A.A.}^0$ is thus

$$n \sum_{i=1}^3 P_{i,A}(p_1)P_{i+\theta}(p_2)/3$$

The reduction rate $\rho_{A.A.1}$ of the evolutionary rate of gene 1 in overlapping expression is, therefore,

$$\rho_{A.A.}^1 = n_{A.A.}^0/n_{A.A.}^N = \sum_{i=1}^3 P_{i,A}(p_1)P_{i+\theta}(p_2) / \sum_{i=1}^3 P_{i,A}(p_1) = (B(\theta) + D(\theta)p_2)/F \quad (3)$$

Similarly, the reduction rate of synonymous change rate is given by

$$\rho_{\text{syno}}^1 = n_{\text{syno}}^0/n_{\text{syno}}^N = \sum_{i=1}^3 P_{i,S}(p_1)P_{i+\theta}(p_2) / \sum_{i=1}^3 P_{i,S}(p_1) = (A(\theta) + C(\theta)p_2)/E \quad (4)$$

Abbreviations used in equations (3) and (4) are

$$v_{\text{syno}}(i) = f_i(0), \quad v_{A.A.}(i) = \sum_{d \neq 0}^{d_c} f_i(d)(1-d/d_c) \quad (5)$$

$$E = \sum_{i=1}^3 v_{\text{syno}}(i), \quad F = \sum_{i=1}^3 v_{A.A.}(i) \quad (6)$$

$$A(\theta) = \sum_{i=1}^3 v_{\text{syno}}(i) v_{\text{syno}}(i+\theta)$$

$$B(\theta) = \sum_{i=1}^3 v_{A.A.}(i) v_{\text{syno}}(i+\theta)$$

$$C(\theta) = \sum_{i=1}^3 v_{\text{syno}}(i) v_{A.A.}(i+\theta)$$

$$D(\theta) = \sum_{i=1}^3 v_{A.A.}(i) v_{A.A.}(i+\theta) \quad (7)$$

From the present definition for the relative shift of reading frame in overlapping genes, the reduction rate of the evolutionary rate for gene 1 can be obtained by putting $\theta = 1$ in equations (3) and (4), and that for gene 2 can be obtained by putting $\theta = 2$ and by exchanging p_1 and p_2 . It can easily be shown that $A(1) = A(2)$, $D(1) = D(2)$, $B(1) = C(2)$ and $C(1) = B(2)$. Then, it is enough to know only the coefficients for the case $\theta = 1$. From this phase-coefficient relationship, ρ_{syno} and $\rho_{A.A.}$ are expressed as follows:

$$\rho_{A.A.}^1 = (B + Dp_2)/F, \quad \rho_{A.A.}^2 = (C + Dp_1)/F \quad (8)$$

$$\rho_{\text{syno}}^1 = (A + Cp_2)/E, \quad \rho_{\text{syno}}^2 = (A + Bp_1)/E \quad (9)$$

Both $\rho_{A.A.}$ and ρ_{syno} are independent of parameter p of their own gene and depend only on that of the other gene. Coefficients A, B, C and D in equations (7) can also be calculated without assuming the independence (exact calculation).

Table 1 shows the values of coefficients of random chains calculated by both the exact and approximate methods, and those of $\Phi X174$ overlapping genes by exact calculation. There is excellent agreement between the results of the two methods. By inserting these values in equations (8) and (9), the reduction in rates of evolution can be estimated.

Figure 1 shows $\rho_{A.A.}$ and ρ_{syno} for each gene as functions of the other gene parameter p . As this figure shows, if evolutionary rates are estimated by the number of substituted amino acids, they are not reduced to such a large extent. That is, the reduction rates are, at most, 1/5 and 2/5 for gene 1 and gene 2 respectively, even if the other overlapping gene allows only synonymous changes. On the other hand, the variability of synonymous changes is considerably reduced in both gene 1 and gene 2 if the genes have small p values. The essential difference between $\rho_{A.A.}$ and ρ_{syno} is caused because A has a relatively smaller value compared with B and C . The difference between $\rho_{A.A.}^1$ and $\rho_{A.A.}^2$ results from the difference between B and C . These features can be qualitatively understood from Fig. 2, which shows the frequency distributions of the amino acid distance caused by a mutation and the rate of acceptance of the mutation at that distance (shaded area) for each base position of codons. Roughly speaking, synonymous changes are allowed only at the third base position, whereas a mutation causing an amino acid change is accepted at a relatively large probability if it arises at the first position, but not the second position. Mutations on the third position seldom cause amino acid changes, or, if changes occur, they are almost always excluded. Then, from equation (7), $A (= A(1))$ is nearly equal to zero, and $B \sim v_{A.A.}(2) \times v_{\text{syno}}(3)$ and $C \sim v_{\text{syno}}(3) \times v_{A.A.}(1)$, so that $C > B$. The parameter p can be estimated from the observed ratio f_0 of the number of nucleotide substitutions causing synonymous changes to the total number of nucleotide substitutions

observed in homologous cistrons. The expected value of this ratio is given by

$$\frac{\sum_{i=1}^3 P_{i,S}(p)}{\sum_{i=1}^3 P_i(p)}$$

Then, we have $p = (E/F)(1 - f_0)/f_0$.

Table 2 shows values of f_0 in translated regions of various cistrons. Although we have insufficient data for firm conclusions, the mean value of f_0 for cistrons listed in Table 2 is 0.79. By using this value of f_0 , we obtain 0.22 for p of our random sequence and 0.18 and 0.20 for p of the *A-B* and *E-D* overlapping genes of Φ X174 respectively. Relatively large values for $\rho_{A,A}$ are obtained in gene 2 of the random sequence and in the *B* and *D* genes of Φ X174—0.44, 0.37 and 0.50 respectively. The $\rho_{A,A}$ in gene 1 of the random sequence and in the *A* and *E* genes of Φ X174 have values 0.25, 0.24 and 0.22 respectively. In contrast, the variability of synonymous changes is considerably reduced. That is, values of ρ_{syno} are 0.15, 0.09 and 0.14 in genes 1, *A* and *E* respectively, and are 0.10, 0.06 and 0.06 in genes 2, *B* and *D* respectively. Although the G-C content and amino acid contents of our random sequence are different from those of the overlapping genes in Φ X174, the respective reductions in variability are consistent with each other. We therefore conclude that, once overlapping gene expression is established, the variability of synonymous changes is considerably reduced, but that of amino acid changes is reduced to a lesser extent. In particular, the reduction of amino acid changes in overlapping genes such as *B* gene and *D* gene in Φ X174 are, at most, 40 or 50%.

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Genetic structure of the East African domestic populations of *Aedes aegypti*

THE yellow fever mosquito, *Aedes aegypti*, is a complex species. Morphological, physiological, and behavioural variation is extensive and complex^{1–4}. In the Rabai area near Mombasa, Kenya, three forms of *A. aegypti* can be distinguished⁵. A light coloured population breeds exclusively in water jars stored inside houses; this is referred to as the domestic form. In disturbed areas adjacent to villages (such as farmland), a form of varying darkness is found breeding in discarded containers or footholds cut in coconut palms; this is the peridomestic form. In tropical forests, an exclusively dark form is found breeding in tree-holes or occasionally rock-holes; this is the feral form. These forms are behaviourally distinct from one another⁵; and, even though they can be found in close proximity to each other, they are often also genetically distinct⁶ (W.J.T., L. E. Munstermann and J.R.P. in preparation). We present here a population genetic analysis of the domestic populations of *A. aegypti* in villages in the Rabai area. We conclude that the mosquito population in each village is a panmictic unit with no significant genetic substructuring; that significant genetic differences exist between villages less than 2 km apart; and that gene frequencies are temporally stable.

Villages in Rabai consist of 10–40 mud-filled frame-walled houses with palm-thatched roofs, clustered together quite closely. Water is stored indoors in 10–20 litre pots. The land between villages consists of small corn plots, coconut groves

or open grassland. Two villages, Mgandini and Majengo, which are 2 km apart, were studied intensively; a third village Kwa Bendegwa, lies between the other two villages and has been studied less extensively.

Genetic variation in *A. aegypti* was studied using gel electrophoresis of enzymes from individual adult mosquitoes. Esterases were assayed from larvae, using an inhibitor⁷. (Details will be published elsewhere.) We have studied 23 enzyme loci; genetic analysis of the electrophoretic variation and genetic mapping of the polymorphic enzyme loci have already been carried out (ref. 8 and L. E. Munstermann, J. M. Lichtenfels and W.J.T., unpublished). We present here data for four polymorphic loci: *Est-6* (coding for an esterase), *Idh-2* (coding for isocitrate dehydrogenase EC 1.1.1.4), *Pgm* (coding for phosphoglucumutase, EC 2.7.5.1), and *Mdh* (coding for malic dehydrogenase, EC 1.1.1.37). These loci are all on the second chromosome, the two outside markers; *Est-6* and *Mdh*, being about 75 map units apart.

Table 1 presents the house by house genotype frequencies at *Est-6*. *F* values indicate deviations using the Hardy–Weinberg rule; positive *F* values are due to excesses of homozygotes; negative *F* values are due to excesses of heterozygotes⁹. On the surface, the positive *F* values indicate that inbreeding occurs in houses due to small population size and restricted migration. If inbreeding is occurring in houses, then in the absence of selection, all loci should show similar *F* values. *Idh-2*, *Pgm* and *Mdh* show no significant deviation from the Hardy–Weinberg equilibrium within any house. Furthermore, as indicated in Table 2, total genotype frequencies in villages conform remarkably well to Hardy–Weinberg expectations.

Three explanations can account for the different behaviour of *Est-6* compared with the other loci. First, selection may act against heterozygotes. This would lead to unstable equilibria, which is unlikely considering the ubiquity of temporally stable genetic variation at this locus (Table 3 and ref. 7). Alternatively, inbreeding may be affecting all loci, with selection in favour of heterozygotes at *Idh-2*, *Pgm*, and *Mdh* compensating for the inbreeding effects. This is unlikely, as selection would have to operate at each locus so as to bring the genotype frequencies into almost perfect Hardy–Weinberg proportions.

The most likely explanation is that there are 'null alleles' at the *Est-6* locus, that is, alleles which show no enzyme activity when stained in our gel system. Heterozygotes with such alleles would be misclassified as homozygotes. Saul *et al.*⁷ reported null alleles at this locus and we occasionally observed 'blanks' on our gels, which may have represented individuals homozygous for null alleles. Thus, electrophoretic studies on *Est-6* alone cannot be used to deduce the population genetic structure of this species.

The other three loci (at which there are no indications of null alleles) are more useful in determining population genetic structure. All three loci indicate no genetic subdivision within a village. We conclude that each Rabai village population of *A. aegypti* is a panmictic unit.

Table 3 summarises results of collections taken at three times over a year in Majengo and Mgandini, as well as one collection from the village of Kwa Bendegwa. These data indicate that Majengo and Mgandini are genetically quite stable over at least a year. Only *Pgm* in Mgandini gives a statistically significant heterogeneity chi-square ($\chi^2 = 19.1$, 4d.f., $P < 0.005$).

The data in Table 3 show the genetic distinctness for each village, despite their close proximity (≤ 2 km). χ^2 analyses show significant gene frequency differences between the three villages (Table 3, sampling *b*) at *Est-6* ($\chi^2 = 52.2$, 12d.f., $P < 0.005$) and *Pgm* ($\chi^2 = 24.3$, 8d.f., $P < 0.005$). Our data indicate that the differences in *Est-6* gene frequencies would not be affected by scoring null heterozygotes as active homozygotes in both populations. To account for the deviations from the Hardy–Weinberg equilibrium in both populations at *Est-6*, the null allele frequencies must be different. Using

Table 1 Observed genotype proportions at *Est-6* in individual houses at Majengo and Mgandini villages with Wright's Inbreeding Coefficient (*F*)

<i>Est-6</i> genotypes													
House no.	<i>n</i>	93/93	97/97	100/100	105/105	93/97	93/100	93/105	97/100	97/105	100/105	Others	<i>F</i>
Majengo													
6	60	1	3	44	0	2	0	1	6	1	2	0	0.419†
7	101	2	1	77	0	1	9	1	5	2	3	0	0.247†
8	59	1	1	35	0	0	10	0	6	2	4	0	0.072
12	141	0	0	116	1	1	3	0	12	5	3	0	0.187*
14	100	1	2	72	0	1	5	1	12	0	6	0	0.149
15	50	0	0	32	0	1	3	0	8	0	6	0	—0.078
16	64	1	3	40	0	0	3	0	9	2	6	0	0.206
18	75	0	4	38	0	2	4	0	16	2	9	0	0.066
28	129	2	6	81	2	1	6	1	11	2	17	0	0.270†
Total	779	8	20	535	3	9	43	4	85	16	56	0	0.192†
Mgandini													
2	46	4	1	10	7	2	3	1	2	3	13	0	0.245
7	45	2	1	12	4	2	7	7	0	1	9	0	0.151
8	90	10	0	29	11	1	14	8	1	0	15	1	0.308†
10	82	11	0	22	18	1	8	9	5	1	6	1	0.452†
17	68	9	0	26	9	2	10	4	1	0	7	0	0.664†
18	72	5	3	21	8	6	7	10	0	4	8	0	0.319†
Total	403	41	5	120	57	14	49	39	9	9	58	2	0.341†

* $P < 0.05$; † $P < 0.01$.

These samples are larvae taken directly from the water pots in each house on 27 December 1976. *n* is the total number of individuals sampled. Expected genotype frequencies were calculated according to Levene's¹³ correction for small sample size. In this table, as well as in Tables 2 and 3, alleles are designated by numbers which indicate their relative electrophoretic mobility.

Table 2 Observed genotype numbers at three loci at Majengo and Mgandini

Genotype	<i>Idh-2</i>		Genotype	<i>Mdh</i>		Genotype	<i>Pgm</i>	
	Majengo	Mgandini		Majengo	Mgandini		Majengo	Mgandini
100/100	59	106	86/86	0	0	100/100	281	234
116/116	93	57	100/100	198	146	123/123	0	7
100/116	151	160	116/116	10	32	130/130	0	1
Others	1	0	86/100	26	4	100/123	24	43
			86/116	4	0	100/130	1	35
			100/116	69	141	123/130	1	0
						Others	0	3
<i>n</i>	304	323		307	323		307	323
χ^2	0.018	0.050		2.190	0.057		0.129	2.409

χ^2 tests for deviation from Hardy-Weinberg expectations based on Levene's¹³ formula for small sample size. *n*, Total number of individuals sampled. None of the χ^2 is statistically significant at the 95% confidence level. These samples are adults reared from the same larval collections for which data are presented in Table 1.

Table 3 Allele frequencies in samples collected at different times from three villages in East Africa

Locus	Majengo				Mgandini		Kwa Bendegwa	
	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>b</i>	<i>b</i>
<i>Est-6</i>	93	0.019	0.012	0.046	0.167	0.144	0.228	0.146
	97	0.019	0.024	0.096	0.026	0.105	0.052	0.056
	100	0.796	0.854	0.805	0.421	0.316	0.443	0.556
	105	0.167	0.110	0.053	0.342	0.409	0.274	0.244
	Others	—	—	—	0.044	0.026	0.002	—
	<i>n</i>	54	82	1,558	114	76	806	82
<i>Idh-2</i>	100	0.469	0.489	0.442	0.512	0.523	0.576	0.603
	116	0.531	0.511	0.554	0.488	0.477	0.424	0.397
	Others	—	—	0.002	—	—	—	—
	<i>n</i>	64	94	608	86	132	646	126
<i>Mdh</i>	86	0.078	0.057	0.049	0.055	0.078	0.006	0.065
	100	0.766	0.670	0.800	0.722	0.655	0.676	0.725
	116	0.141	0.273	0.151	0.222	0.267	0.317	0.210
	<i>n</i>	64	88	614	72	116	614	124
<i>Pgm</i>	100	0.984	0.978	0.956	0.907	0.893	0.845	0.914
	123	0.016	0.022	0.041	—	0.107	0.088	0.023
	140	—	—	—	0.070	—	—	0.063
	Others	—	—	0.003	0.033	—	0.067	—
	<i>n</i>	64	92	614	86	122	646	128

Est-6 frequencies may not be precise because of the presence of null alleles discussed in the text. *a*, Sample taken in November 1975; *b*, April 1976; *c*, December 1976. Samples *a* and *b* are based on *F*₁ progeny from field collections; *c*s are field caught individuals. *n*, Number of genes sampled from natural populations.

the direct relationship between Wright's inbreeding coefficient (F) and the deficiency in observed heterozygotes, we calculated the expected frequency of null alleles needed to conform frequencies to Hardy-Weinberg equilibrium. We found that Majengo and Mgandini (Table 3, sampling *c*) significantly differ with expected null allele frequencies of 0.106 ± 0.0002 and 0.206 ± 0.0007 , respectively. All four loci showed significant gene frequency differences between the extensive samples from Majengo and Mgandini (Table 3, sampling *c*), *Est-6* ($\chi^2 = 487.8$, 4d.f., $P < 0.005$), *Idh-2* ($\chi^2 = 24.9$, 2d.f., $P < 0.005$), *Mdh* ($\chi^2 = 62.4$, 2d.f., $P < 0.005$) and *Pgm* ($\chi^2 = 50.6$, 3d.f., $P < 0.005$).

These results indicate that gene flow between villages is not frequent enough to prevent genetic differentiation due to selection or random genetic drift. Further, this implies that the surrounding field-breeding outdoor *A. aegypti* do not act as a genetic bridge between adjacent populations of indoor breeding *A. aegypti*. Mark-recapture studies confirm this¹⁰.

It has often been proposed that genetic manipulation of pest insect species may be helpful in suppressing population sizes or rendering species harmless¹¹. In order to effectively carry out such programmes, the population genetic structure of the target species should be known. Rabai village populations of *A. aegypti* are stable panmictic units with very limited gene flow from outside the village. These populations have about 500–1,000 adults in each village¹² and therefore are of a nature which would make genetic control possible. However, the genetic individuality of each village population may cause other problems. If a release programme for population suppression or replacement is successful in one village, releasing the same material in a village less than 2 km away may not succeed. We hope that this study illustrates the usefulness of modern population genetic techniques in designing control programmes.

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Table 1 Screening of mutagenised clones for auxotrophy

	Clones screened	+	±	—	+	+	±	—	—	±	Rich medium
		+	±	—	±	—	—	±	+	+	Minimal medium
First screening	926	694	12	52	13	125	18	4	3	5	
Second screening	115*	70	5	7	23	8	2	0	0	0	

First screening: clones surviving the mutagenesis (see text) were transferred with the flat ends of sterile toothpicks into separate wells of Linbro microplates (no. IS-FB-96) containing 0.1 ml of 17 mM K/Na₂-phosphate, pH 6.0 (Sorensen's buffer) and dihydrostreptomycin-sulphate (0.2 mg ml⁻¹). Each of these master plates was replicated twice with a multipronged device⁸, once into minimal medium (0.1 ml per well) and once into rich medium (0.1 ml per well) in Linbro microplates (no. IS-MRC-96). Rich medium contained all the components of minimal medium supplemented with: alanine, 0.2 g l⁻¹; aspartic acid, 0.3 g l⁻¹; glutamine, 0.5 g l⁻¹; serine, 0.2 g l⁻¹; tyrosine, 0.4 g l⁻¹; adenosine, 0.2 g l⁻¹; uridine, 0.2 g l⁻¹; thymidine, 0.1 g l⁻¹; torula yeast RNA, 0.5 g l⁻¹; herring sperm DNA, 0.02 g l⁻¹; pyridoxin·HCl, 0.25 mg l⁻¹; pyridoxal·HCl, 0.25 mg l⁻¹; Ca-pantothenate, 1.5 mg l⁻¹; ascorbic acid, 4 mg l⁻¹; inositol, 4 mg l⁻¹; para-aminobenzoic acid, 2.6 mg l⁻¹; nicotinic acid, 8 mg l⁻¹; indole acetic acid, 5 mg l⁻¹; and haematin, 0.25 mg l⁻¹. The replicated colonies were incubated at 21–22°C in the dark and scored for growth after about 4 weeks. Colonies were scored + (good growth), ± (poor growth), or — (no visible growth). All wells which scored + in rich medium but — in minimal medium were cloned on bacterial lawns and stored at 4°C. Second screening: clones were retested by transferring cells from individual clones to wells of Linbro trays (no. FB-16-24-TC) containing 0.8 ml of rich medium. These trays were incubated at 22°C on an orbital shaker at 150 r.p.m. After the cells had grown, the well contents were collected by centrifugation, washed once with 5 ml of Sorensen's buffer and resuspended in 1 ml of minimal medium. These cells were diluted to 8×10^4 to 2×10^5 per ml in 5 ml of minimal or rich medium in 50 ml Erlenmeyer flasks, and incubated at 22°C on an orbital shaker (120 r.p.m.).

*Scored + in rich medium, — in minimal medium in first screening.

of selectable markers. The creation of a defined minimal medium³ has made possible the isolation of auxotrophic mutants which we describe here. These auxotrophs should improve linkage analysis by increasing the number of selectable genetic loci and should also prove useful for the selection of diploids and for a variety of biochemical purposes.

All auxotrophs were isolated in an axenic strain, HH25, which is a temperature-sensitive mutant derived from strain AX3. HH25 grows normally at 22°C but does not grow at 26.5°C. It was mutagenised with *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine⁴ such that 1.7% of the amoebae were recovered as clones growing on lawns of *Klebsiella aerogenes*⁵. These survivors were tested for auxotrophy as described in the legend to Table 1. Of 926 clones originally transferred to microtitre wells, 706 grew in both media and 125 grew only in the rich medium. In addition, there were smaller classes which grew better in one medium than the other, or not at all, as listed in Table 1. Of the 125 potential auxotrophs, 115 were rescreened and most (70) proved to be non-auxotrophs, while 8 continued to grow well in rich medium and not at all in the minimal medium. These remained auxotrophic in all subsequent tests, and their growth requirements have now been determined.

Table 2 lists the nutritional requirements of these strains. HH32 is apparently a simple pyridoxin-requiring strain. The pyrimidine-requiring strains HH33 and HH34 are not identical because the first can utilise orotate and dihydroorotate, while the second can only utilise orotate. Strain HH34 is probably affected in the enzyme dihydroorotate dehydrogenase, while HH33 carries a mutation affecting the *de novo* pathway for pyrimidine biosynthesis before the synthesis of dihydroorotate; the enzyme dihydroorotase is

Auxotrophic mutants of *Dictyostelium discoideum*

THE cellular slime mould *Dictyostelium discoideum* is a unicellular organism in its vegetative stage. On starvation, slime mould amoebae gather into aggregates which pass through a morphogenesis culminating in mature fruiting bodies consisting of dead stalk cells supporting a sphere of viable spores¹. The laboratory strains of *D. discoideum* are normally haploid, and the genetic techniques available to study cellular differentiation are based on a parasexual system of analysis which includes selection of temperature-resistant diploids after fusion of two haploid strains bearing complementing temperature-sensitive mutations. Recovery of rare haploid segregants is accomplished by selecting recessive drug resistance markers incorporated into one of the parents². The parasexual system suffers from a dearth

Table 2 Nutritional requirements of auxotrophs

Strain name	Mutation name	Growth*	No growth*
HH32	pdx-300	Pyridoxin-HCl (0.5 µg ml ⁻¹) Pyridoxal-HCl (0.5 µg ml ⁻¹)	— —
HH33	pyr-300	Na-orotate (0.13 mg ml ⁻¹) Uracil (0.2 mg ml ⁻¹) Uridine (0.2 mg ml ⁻¹) Cytidine (0.4 mg ml ⁻¹)	Carbamylphosphate† (0.4 mg ml ⁻¹) Carbamyl-DL-aspartic acid (0.4 mg ml ⁻¹) L-dihydroorotic acid (0.3 mg ml ⁻¹) Thymidine (0.4 mg ml ⁻¹)
HH34	pyr-301	L-dihydroorotic acid Na-orotate (0.13 mg ml ⁻¹) Uracil (0.2 mg ml ⁻¹) Uridine (0.4 mg ml ⁻¹) Cytidine (0.4 mg ml ⁻¹)	Carbamylphosphate† (0.4 mg ml ⁻¹) Carbamyl-DL-aspartic acid (0.4 mg ml ⁻¹) Thymidine (0.4 mg ml ⁻¹)
HH36 HH39	pur-300 pur-302	Adenine (0.2 mg ml ⁻¹) Adenosine (0.4 mg ml ⁻¹) 5'-AMP (1 mg ml ⁻¹) Cyclic AMP (0.6 mg ml ⁻¹) Hypoxanthine (0.2 mg ml ⁻¹) Inosine (0.4 mg ml ⁻¹)	Xanthine (0.2 mg ml ⁻¹) Xanthosine (0.6 mg ml ⁻¹) Guanosine (0.4 mg ml ⁻¹) Uric acid (0.2 mg ml ⁻¹)
HH37	pur-301	Guanosine‡ (0.2 mg ml ⁻¹) 5'-GMP‡ (0.4 mg ml ⁻¹)	Adenine (0.2 mg ml ⁻¹) Adenosine (0.4 mg ml ⁻¹) 5'-AMP (1.2 mg ml ⁻¹) Xanthine (0.2 mg ml ⁻¹) Hypoxanthine (0.2 mg ml ⁻¹) Inosine (0.4 mg ml ⁻¹)
HH38	—	Amino acids‡§	RNA + DNA§ adenosine + uridine + thymidine§ Vitamins§
HH40	—	Alanine (0.1 mg ml ⁻¹) Aspartic acid (0.15 mg ml ⁻¹) Glutamine (0.25 mg ml ⁻¹) Serine (0.1 mg ml ⁻¹)	RNA + DNA§ Adenosine + uridine + thymidine§ Vitamins§

*Cells were collected by centrifugation from rich medium, washed once with Sorensen's buffer, and resuspended in minimal medium with supplements. Initial cell densities were 10^5 – 2×10^5 cells per ml.

†Carbamylphosphate was added with and without the addition of L-aspartic acid (0.3 mg ml⁻¹).

‡Stimulated growth, but not to the level obtained in rich medium.

§Concentrations as in Table 1 legend.

lacking (Table 3). We do not know whether the mutation is in this particular enzyme or whether carbamylphosphate synthetase and aspartate transcarbamylase are also affected. It is known that in other systems these enzymes are co-ordinately regulated and form one multi-enzyme complex^{7,8}.

Of the purine-requiring strains, HH36 and HH39 grow with hypoxanthine or adenine as well as with their ribonucleosides and ribonucleotides. Growth of strain HH37 is stimulated by guanosine or 5'-GMP, but for optimal growth the complete rich medium is required.

HH38 and HH40 grow when presented with a mixture of non-essential amino acids, but any single amino acid does not stimulate growth. Single omission experiments demonstrated that HH40 requires alanine, aspartic acid, glutamine and serine for optimal growth. HH38 requires other un-

identified components of the rich medium for optimal growth. HH37, HH38, and HH40 may be multiple auxotrophs; if this is the case, it should be possible to derive singly auxotrophic haploid strains after segregation from diploids.

The morphogenesis following aggregation of the auxotrophic strains is incomplete. When grown on bacterial lawns, strains HH33, HH37 and HH40 do not form spores; all other strains do form spores when grown on bacterial lawns. When starved on filters⁵, aggregates of strain HH33 arrested uniformly in club-shaped structures at a stage of development before the completion of spores. All other strains produced a variety of final structures, including some fruiting bodies.

In no case was it possible to rescue strains developing on filters with exogenous nutrients. Whether this inability to complete the last stages of development is due to the various auxotrophies or to secondary mutations is not clear.

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Table 3 Dihydroorotase activity of mutant and wild-type strains

Strain	Dihydroorotase (EC3.5.2.3) (units per mg protein)
AX3 (wild type)	49
HH25	45
HH33	< 3
HH34	27

Strains were grown to a density of 7×10^6 cells ml⁻¹ in HL/5 (ref. 3) supplemented with uridine (0.3 mg ml⁻¹), collected by centrifugation, washed once with Sorensen's buffer, and resuspended in 10 mM Tricine-NaOH (pH 7.6), 10 mM KCl, 0.1 M sucrose to a density of about 5×10^7 cells ml⁻¹. The cell suspension was frozen at -20 °C, thawed, and centrifuged (30 min at 40,000g). The supernatant was assayed for activity by adding 25–50 µl to 1 ml of reaction mixture preincubated at 37 °C. A_{230nm}^{3cm} was measured at 37 °C in a Beckman 25 double-beam spectrophotometer. 1 ml reaction mixture contained: 100 µmol K₂ phosphate (pH 6.0), and 10 µmol carbamyl-DL-aspartate. The reference cell lacked carbamyl-aspartate. One unit equals the conversion of 1 nmol carbamyl-aspartate to dihydroorotate per min. ϵ_{230nm}^{3cm} (dihydroorotate) = 1.17×10^3 . Protein was determined by the Lowry method⁹ using bovine serum albumin as the standard.

Rescue of a lethal T/t locus genotype by chimaerism with normal embryos

THE mutation T^{Hp} at the T/t locus in the mouse is unique among mammalian genes because it has a sharply defined maternal effect. Like other known T/t locus dominant mutations, T^{Hp} yields a short-tailed phenotype in heterozygotes of both sexes. However, $T^{Hp}/+$ females when mated with wild type fail to give birth to short-tailed progeny; examination of embryos shows that heterozygous embryos die late in gestation with seemingly minor defects such as spina bifida and polydactyly¹⁻³. The precise reason for death has not been determined, but must depend on some defect intrinsic to the egg, because $T^{Hp}/+$ females successfully carry to term heterozygous embryos whose T^{Hp} gene is paternally derived. I report here that lethal $T^{Hp}/+$ maternal heterozygotes are readily rescued if they are aggregated with normal embryos, but that the germ cell defect persists, because the chimaeras fail, if female, to transmit T^{Hp} to viable offspring.

Female $T^{Hp}/+$ heterozygotes from our own stock that were black-and-tan and known to be homozygous CC were crossed, after superovulation, to a random-bred Swiss albino stock (ICR) obtained commercially (Camm Research). Embryos recovered from these females 2 d after mating were approximately 8–16 cells, and were aggregated in pairs with comparable embryos from ICR $\times$ ICR matings according to described procedures⁴ modified in accordance with practical instruction in chimaera formation kindly provided by V. E. Papaioannou. After 24 h of growth *in vitro*, well aggregated morulae and/or blastocysts were transplanted to pseudo-pregnant ICR hosts. Nineteen living young were obtained; their descriptions and breeding records are given in Table 1.

Sixteen animals were coat-coloured chimaeras. Six of these were phenotypically short-tailed, thus demonstrating simultaneously that T^{Hp} is expressed in chimaeras, and that embryos obtaining T^{Hp} from their mother can be rescued by chimaerism with wild-type embryos. The degree of expression is reduced, however; only two of the six obvious short-tailed animals had tails scored as 2/3–3/4 normal length, which is in the range for ordinary $T^{Hp}/+$ animals. The others had only minor shortening or blunting of the tail tip; one male scored as normal tailed transmitted T^{Hp} to progeny and so was clearly a $T^{Hp}/+ \longleftrightarrow +/+$ chimaera. $T^{Hp}/+$ embryos seem not to be compromised in their ability to participate in the formation of chimaeras, because of the 16 coat-colour chimaeras obtained, seven (very close to the

1/2 expected) contained the $T^{Hp}/+$ genotype. Likewise, the degree of coat colour chimaerism was not noticeably different in normal compared to short-tailed animals, thus indicating that $T^{Hp}/+$ cells are as competent as normal cells to contribute to that somatic tissue.

All three short-tailed males (and the one phenotypic normal mentioned above) produced substantial numbers of short-tailed progeny. None of the three female chimaeras, however, produced any offspring carrying T^{Hp} , although all produced large numbers of pigmented progeny that demonstrated the production of oocytes derived from the $T^{Hp}/+$ component. Because the $T^{Hp}/+$ component in these chimaeras is C/c , only inferences can be made about whether oocytes are also being produced by the ICR component. Because of the nature of sex differentiation in chimaeras, most phenotypic females are genotypically $\varnothing \longleftrightarrow \varnothing$ (ref. 5), so the most likely situation is that the ovaries of these animals contain both $T^{Hp}/+$ and $+/+$ oocytes. Thus, it seems that neither somatic chimaerism nor like-sex gonadal chimaerism is able to restore normality to eggs that receive the T^{Hp} gene from the mother.

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A stereometric pattern of distribution of acetylthiocholinesterase in the deep layers of the superior colliculus

THE superior colliculus, although a layered structure, is not classified as a cortex because most of its neurones lack long apical dendrites. The colliculus, nevertheless, seems to share with the neocortex some of the features of columnar organisation. In particular, a periodic segmentation of certain afferent connections has been found, in which incoming fibres are arranged in the colliculus in bands or slabs, much like the ocular dominance columns in the striate cortex¹⁻⁶. The possibility that such a distribution pattern might be evident also in a histochemical analysis of the superior colliculus was raised by Ramon-Moliner's finding⁷, in a survey of acetylthiocholinesterase activity in the cat's brain stem, that this enzyme is distributed in a series of regularly spaced clumps in the deep collicular layers. Evidence is presented here that such enzyme-positive clumps are also seen in the monkey and that, in the cat at least, these clumps do indeed form longitudinal bands.

Observations were made in two Rhesus monkeys, five cats and one kitten aged 3 weeks. With one exception (adult cat), brains were fixed by mixed-aldehyde perfusion. Sections cut at 30–50 μ m on a freezing microtome were treated according to the protocols either of Karnovsky and Roots⁸ or of Geneser-Jensen and Blackstad⁹, usually with the addition of an inhibitor of nonspecific cholinesterases (ethopropazine). Control sections from one cat were incubated: in the absence of the acetylthiocholine iodide substrate; with butyrylthiocholine iodide; by the addition of eserine to the normal incubation medium (10^{-3} – 10^{-5} M). One brain was perfused with saline only and blocked in the mid-sagittal plane. Half of the brain stem was processed immediately, without fixation; the other half was soaked in fixative for 2 d before being cut and stained.

Table 1 Phenotypic expression and breeding behaviour of 19 mice derived from morulae from $\varnothing T^{Hp}/+ \times \sigma$ ICR parents aggregated with morulae from $\varnothing$ ICR $\times$ σ ICR matings

Sex	Coat colour (% albino)	Tail phenotype	Offspring $\times$ ICR			
			Normal tail		Short tail	
			Albino	Pigmented	Albino	Pigmented
$\varnothing$	100	Normal	25	—	—	—
$\varnothing$	100	Normal	14	—	—	—
$\varnothing$	100	Normal	10	—	—	—
$\varnothing$	50	Normal	6	1	—	—
$\varnothing$	5	Normal	5	24	—	—
$\varnothing$	50	2/3	11	17	—	—
$\varnothing$	50	9/10	38	28	—	—
$\varnothing$	50	9/10	36	38	—	—
σ	98	Normal	27	—	—	—
σ	95	Normal	53	—	—	—
σ	50	Normal	37	23	—	—
σ	50	Normal	45	24	—	—
σ	50	Normal	36	—	—	—
σ	60	Normal	32	—	—	—
σ	50	Normal	31	22	—	—
σ	50	Normal	17	15	17	15
σ	75	Blunt	50	5	2	4
σ	10	9/10	26	26	26	16
σ	40	3/4	16	14	16	14

Dense patches of acetylthiocholinesterase activity were seen in the intermediate grey layer of the posterior half of the superior colliculus in all brains examined. These enzyme-positive patches were about 200 to 400 μm wide and were separated by sparsely stained zones. In the kitten, where the patches seemed to be most evenly distributed (Fig. 1a), they lay just beneath the stratum opticum, were spaced at fairly regular intervals and in occasional sections showed a systematic lateral-to-medial increase in diameter indicative of considerable orderliness. In the adult cat (Figs 1b,c) the enzyme deposits often appeared as wisps of irregular shape and, though arranged in a chain-like sequence across the width of the intermediate grey layer, the patches tended to fuse with one another or to send streamers into deeper strata. Especially in the medial half of the colliculus, the main chain of clumps was associated with a variable number of enzyme patches in deeper collicular tiers and with a region of prominent staining in the dorsolateral central grey substance (Fig. 1c).

In the Macaque monkey, as in the cat, alternating regions of high and low cholinesterase activity were observed in the upper part of the intermediate grey layer, but there was considerable variability in the size and shape of the enzyme patches, and in one of the monkeys (Fig. 1d) they locally

formed two tiers along the upper and lower margins of the intermediate grey layer. Neither in the monkey nor in the cat did the presence or absence of a nonspecific cholinesterase inhibitor, or choice of histochemical protocol^{8,9} seem to alter the basic clumping pattern of the enzyme deposits; the omission of fixation (cat only) also failed to produce a markedly different distribution. The enzyme-positive patches were not seen in control sections processed in the presence of eserine or in sections incubated with butyrylthiocholine iodine.

To determine the pattern of acetylthiocholinesterase distribution in further detail, a planar reconstruction was assembled from serial transverse sections through the cat superior colliculus. Figure 2 shows the dorsal view of the enzyme-positive regions that results from registering the patches as straight line-segments on a flattened representation of the intermediate grey layer^{2,3}. It is evident from this map that the clumps seen in individual cross-sections are continued from section to section with preserved periodicity, and that they are actually arranged in narrow bands. In Fig. 2 the banding pattern is more striking in the right colliculus than in the left, where several of the bands seem to emerge from a major anastomotic node near the caudal pole of the colliculus. There are indeed, on

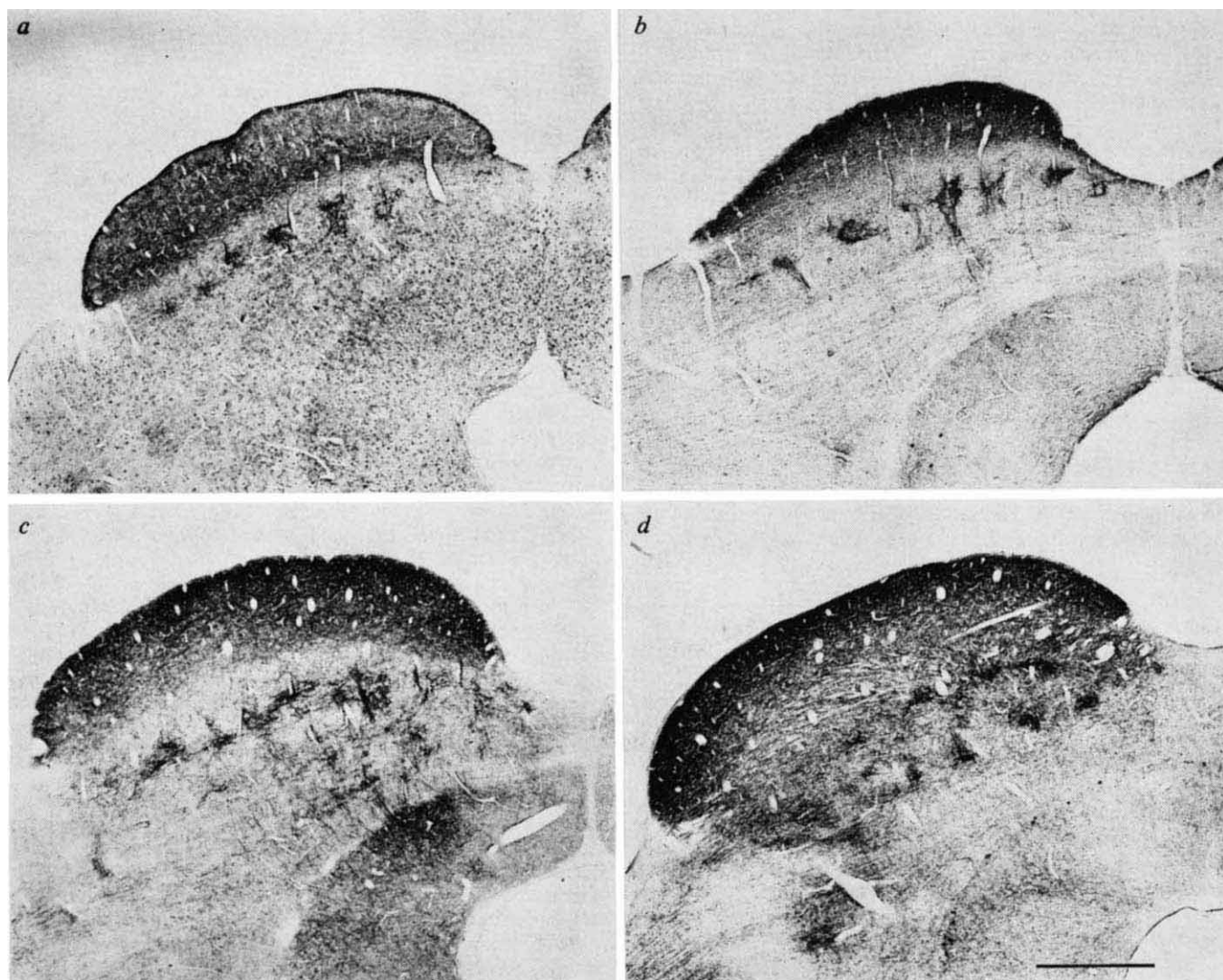


Fig. 1 Photomicrographs showing acetylthiocholinesterase activity (dark stain) in transverse sections through the superior colliculus of 3-week-old kitten (a), adult cat (b, c) and adult monkey (d). Histochemical processing by Karnovsky-Roots (b) and Geneser-Jensen and Blackstad (a, c, d) protocols shows sharp contrast between relatively dense, uniform distribution of enzyme in the superficial grey layer and segmented chain of enzyme patches in the intermediate grey layer. Two tiers of enzyme patches prominent in monkey (d), tiers also visible in cat (c). Regular lateral-to-medial increase in clump diameter in kitten (a) should be compared with reverse pattern often seen in nigro-tectal clump distribution³. Bar indicates 1 mm.

both sides, numerous irregularities in the pattern; few bands extend without interruption through most of the enzyme-product's distribution zone. There is, nevertheless, a clear impression of a series of stripes or bands disposed in a predominantly longitudinal direction.

Only one serial-section reconstruction has been completed, but certain characteristics of the distribution of acetylthiocholinesterase in the colliculus seem to hold true for all animals studied. First, the clumps are most prominent in the caudal half of the colliculus and fade rapidly toward the rostral pole. Although localised for the most part in the upper part of the intermediate grey layer, the enzyme deposits also appear in variable arrangement deeper in the colliculus. This tendency toward stratification, first noticed by Ramon-Moliner in the cat⁷, was especially prominent in the present series in one of the two monkeys (Fig. 1d). Finally, although the cholinesterase content of the colliculus is apparently highest in the superficial grey layer, where the enzyme has long been known to occur, its distribution in that layer shows little if any sign of vertical segmentation, and at most a tendency toward horizontal condensation, especially in the kitten (Fig. 1a).

Although the bands of acetylthiocholinesterase in the superior colliculus do not necessarily represent sites of cholinergic transmission (see, for example, refs 7, 9, 10), this possibility seems well worth further study. For the striking regularity in the distribution of this enzyme, both in the colliculus and, as just recently described¹¹, in the

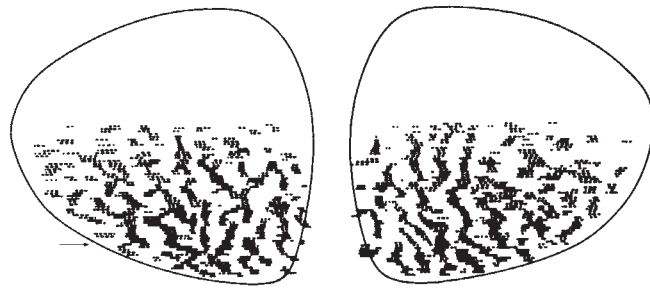


Fig. 2 Dorsal-view reconstruction of acetylthiocholinesterase banding in superior colliculi of adult cat assembled from serial 50- μ m transverse sections. Patches of enzyme activity in intermediate grey layer are registered as horizontal line segments on a flattened representation of the colliculus, solid lines marking dense enzyme product, dotted lines weak enzyme activity. Outlines show borders of superficial grey layer of each colliculus. Arrow on left indicates location of the transverse section shown in photomicrograph of Fig. 1b.

cerebellum as well, could serve as a signature of a particular intrinsic or extrinsic fibre system. In fact, two other sets of longitudinal bands, similar in general form to those reported here, have been shown by autoradiographic fibre tracing to exist in the superior colliculus of the cat. In the superficial grey layer such bands represent locally concentrated distributions of fibres from the ipsilateral retina². In the intermediate grey layer, bands are formed by terminal clusters of nigro-tectal fibres³. It is not clear what structures are being stained by the thiocholinesterase methods used in the present study, and, in particular, there is no reason to be sure that it is the terminal distributions of such fibre systems that are responsible for the clumping. Nevertheless, the marked similarity in distribution of the acetylthiocholinesterase bands and the bands of nigro-tectal fibre termination, both being in the same layer, suggests some relationship (interdigitation, overlap or even identity) between the two. Neocortical sources of clumped deep-collicular afferents, not yet studied in serial-section reconstructions, also need to be considered both in the cat^{3,12} and in the monkey^{13,14}.

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Central neuronal control of cilia in *Tritonia diamedia*

CONSIDERABLE evidence indicates that the beating of cilia of a wide variety of animals is under neural control¹, but no recordings have yet been made of the central neurones which actually control or influence ciliary beating. Several invertebrates of several phyla locomote by means of ciliary beating^{2,3}; among these are included members of several families of gastropods⁴⁻⁷. The pedal cilia of these gastropods must be under central nervous control, but apart from some early experiments demonstrating that central control does exist^{4,5} it has received little attention⁸. I report here that the nudibranch mollusc *Tritonia diamedia* locomotes by pedal ciliary action, and that re-identifiable 'cilia motor neurones' in the central nervous system stimulate the beating of the pedal cilia.

Most gastropods crawl along the substrate by muscular pedal waves^{5,7} or by ciliary action⁴⁻⁶. It is sometimes stated that only animals of very low mass can move using cilia since ciliary forces are very small. Some quite large marine gastropods, however, which are buoyant and whose weight is largely supported by the water, are able to use cilia for crawling^{5,6}. There is no unequivocal experimental test to demonstrate that locomotion is ciliary in nature⁶, since no treatment exists which selectively stops ciliary beating without possible side effects on the snail. The deduction of ciliary locomotion is, therefore, based largely on visual criteria. First, purely ciliary locomotion will occur in the absence of any muscular activity of the foot. Second, the entire sole will adhere closely to the substrate and move forward uniformly and smoothly. Third, the foot is covered with cilia, which beat while the animal is moving and which are quiet when the animal is at rest^{4,5}.

Tritonia diamedia, a large nudibranch mollusc of the North Pacific, shows all of these characteristics during its normal crawling locomotion. In addition, its maximum locomotion rate corresponds very closely to the maximum rate of particle transport by the pedal cilia. If NaCl crystals are dropped on to a *Tritonia*, it performs its 'escape swim'^{9,10}; immediately on terminating the swim, the animal settles to the bottom and begins crawling at what seems to be top speed, measured at 1.9 ± 0.3 mm s⁻¹ (mean $\pm$ s.d.; $n=16$). The rate of transport of carbon black particles on the sole of the foot during salt-induced ciliary beating is nearly the same: 2.1 ± 0.4 mm s⁻¹ ($n=10$). The beating of the pedal cilia are very probably the sole cause of normal crawling locomotion in *Tritonia*. *Tritonia* shares with several other opisthobranchs a number of advantages for investigation of the neural control of behaviour, among

which are large, often repeatably identifiable neurones^{9,11} and the ability and willingness to show apparently normal behaviour patterns in semi-intact preparations while intracellular recordings are made from central neurones^{9,10,12}.

To study the neural control of locomotory cilia, a modification of the semi-intact preparation of Willows^{9,10,12} was used and is described in the legend to Fig. 1. The ventral side of the brain is normally exposed in this way, although careful dissection allows the brain to be flipped over to expose the dorsal side. Many of the normal behaviour patterns of *Tritonia* occur spontaneously or can be elicited in this preparation, including escape swimming, branchial tuft withdrawal, and so on.

A pair of large, repeatably identifiable neurones, one on the ventral side of each pedal ganglion, were found to be 'motor neurones' for excitation of pedal ciliary beating (Fig. 1a). These neurones were termed left and right pedal 21 (L and R Pd 21), respectively, in the numbering system of Willows *et al.*¹². Their identification as motor cells for the cilia was based on several factors. Each sends its axon into the ipsilateral pedal nerve 3 (PdN 3); this nerve innervates the posterior ipsilateral two-thirds of the foot. Axon location was confirmed by the presence of antidromic spikes originating in PdN 3, by cobalt chloride backfills of PdN 3, and by direct intracellular staining with cobalt, which showed

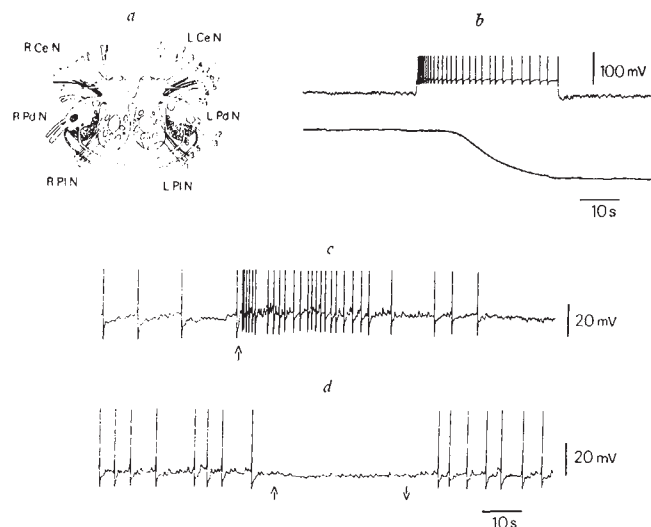


Fig. 1 *Tritonia* preparations were a modification of the semi-intact preparation of Willows^{9,10,12}. *Tritonia* were cooled to near 0 °C and then the anterior one-third of the foot was slit up the midline. The animal was pinned, ventral side up, in a wax-bottomed dish, and the buccal mass was pulled out and forward, and pinned. The buccal ganglia were removed from the buccal mass, and the oesophagus cut, care being taken to leave the cerebrobuccal connectives and the pedal commissures intact. This exposed the brain, composed of paired cerebro-pleural and pedal ganglia, which lies dorsal to the oesophagus. The animal was then unpinned and transferred to a Plexiglas chamber filled with continuously flowing seawater^{9,12}. The animal was held in the chamber, ventral side up, by hooks placed around the edge of the cut foot. The buccal mass was pulled forward by another hook. A stage covered with clear Sylgard resin (Dow Corning) was then manipulated underneath the brain, and the brain pinned to it. *a*, Drawing of the ventral side of the *Tritonia diomedea* brain, the right Pd 21 neurone is hatched. CeN, Cerebral ganglion nerves; PdN, pedal ganglion nerves; P N, pleural ganglion nerves. (Numbering from Willows *et al.*¹².) *b*, Upper trace: Right Pd 21 neurone, impaled with two microelectrodes, one for recording, one for stimulation. Lower trace: photocell aimed at a portion of the posterior right side of the foot. When R Pd 21 is stimulated intracellularly, cilia move the carbon black particles off the foot, exposing the pale pink tissue beneath, which reflects more light to the photocell. *c*, Right Pd 21; stimulation of the tail of the *Tritonia* by a few drops of 1.5 M NaCl at the arrow. *d*, Right Pd 21 (same cell as in *c*); gentle touch of oral veil with a sea whip (*Virgularia* sp.) between the arrows.

an axon emerging out this nerve. Another axon enters PdN 5, a commissure leading to the other pedal ganglion. The two Pd 21 neurones are electrically coupled to one another by way of the PdN 5 axons, and, when combined with normally synchronous synaptic inputs, the coupling helps to coordinate activity in the two sides (my work in preparation).

Intracellular stimulation of a Pd 21 neurone causes ciliary beating to occur over large areas of the posterior portion of the ipsilateral half of the foot, as measured by the movement of carbon black particles (Fig. 1b). The number and frequency of spikes necessary to drive ciliary movement are less than those which occur normally in response to external cilioexcitatory stimuli. This excitation of ciliary activity due to intracellular stimulation still occurs in *Tritonia* in which the brain is bathed in an artificial seawater containing 200 mM Mg²⁺ and 1 mM Ca²⁺, while the rest of the animal is maintained in natural seawater. Chemical synaptic activity is not observed in the brain in this solution, and behaviour patterns which require functional chemical synapses, such as the escape swim, cannot be evoked.

Externally applied stimuli which normally cause an increase in locomotion and in ciliary beating in an intact animal, such as a few drops of 1.5 M NaCl on the tail, increase excitatory synaptic activity and spiking in Pd 21 (Fig. 1c). External stimuli which normally cause a crawling *Tritonia* to stop abruptly, such as the touch of a sea whip (*Virgularia* sp., a favourite food) to the chemosensitive oral veil, cause an inhibition of spiking in Pd 21 (Fig. 1d).

Although the evidence indicates that the Pd 21 neurones act peripherally, this does not mean that they necessarily innervate the cilia-bearing cells of the foot directly. There are three means by which they might stimulate ciliary beating. First, the axons of these neurones might synapse directly on each ciliated cell in the foot, or on some of them, from which excitation could spread to nearby cells. Second, the neurones might release a neurohormone into tissue spaces in the foot. Third, the neurones might innervate the peripheral nerve plexus found in gastropods, and this plexus may in turn excite the ciliated cells.

Neural control of, or influence on, cilia has been proposed in many organisms¹. For example, Mackie *et al.*¹³ concluded from ablation experiments and morphological examination, that the arrest of beating of the pre-oral cilia of gastropod veligers is under neural control. The rate of beating of the lateral cilia of the gill of the bivalve *Mytilus edulis* is altered by stimulation of the branchial nerve¹⁴ or visceral ganglion¹⁴, and is also influenced by pharmacological¹⁵ and surgical¹⁶ manipulations of the central nervous system. The beat frequency of pharyngeal cilia of the frog is also reported to be under nervous control¹. To my knowledge, however, there is no other instance in the literature in which the central neurones which affect ciliary beating have been identified. Since these are large, repeatably identifiable neurones, they provide an excellent opportunity for further study of the neural and pharmacological control of ciliary activity.

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Selection and altered properties of brain-colonising metastatic melanoma

Two of the least understood properties of malignant tumour cells are their abilities to invade surrounding normal tissues and to escape to form secondary tumours (metastases) at near and distant sites¹⁻³. Distant metastases can arise when invading malignant cells penetrate into and are disseminated in the lymphatics, coelomic cavities or blood circulatory system. The mere presence of tumour cells in the blood does not necessarily indicate that distant metastatic disease will follow⁴, for most blood-borne malignant cells die rapidly in the circulation with few surviving to form secondary tumours²⁻⁵. In various experimental blood-borne metastatic tumour systems the location of gross metastases is nonrandom and does not correlate with the first capillary bed encountered⁶⁻¹⁰. This suggests that other factors are involved in tumour cell arrest apart from nonspecific trapping^{2,3,9,10}. These would include cellular adhesive interactions of tumour cells with themselves¹¹⁻¹³ (homotypic aggregation), heterotypic aggregations with circulating¹⁴⁻¹⁶ and non-circulating^{12,17,18} host cells, interactions with blood clotting components¹⁹⁻²¹, tumour cell deformability^{22,23}, release or display of malignant cell hydrolytic enzymes^{24,25} and interactions with host immunological defences^{2,16,18,26,27}. Unique tumour cell surface properties probably determine malignant cell arrest and survival at specific sites^{3,12,17,18,28}, and this has been demonstrated when enzymatic treatment of malignant tumour cells has modified experimental metastasis^{26,29}. We report here that variant cell lines of malignant melanoma can be selected *in vivo*²⁸ for brain-specific colonisation³⁰. These variants have cell surface characteristics that correlate with their brain specificity *in vivo*.

Brain-colonising melanoma lines were selected sequentially from a murine B16 melanoma line (originally called line 26 and now designated B16-F1)²⁸ that was initially selected once for lung implantation, survival and growth by Fidler²⁸. B16-F1 was used first to select for tumour cell lung colonisation by intravenous (tail vein) injection of suspensions of single cells, and 3 weeks later the resulting pulmonary tumour colonies were removed and adapted to tissue culture to form the line B16-F2, which was further selected *in vivo* for lung colonisation. After ten such selections the tumour cell line B16-F10 was obtained; it formed significantly more gross metastases in the lungs than did B16-F1^{15,28} and yielded exclusively lung tumours^{9,10}.

Table 1 Experimental metastases found after intravenous injection of variant B16 melanoma cell lines

Melanoma line	No. of brain tumours per mouse	Median (range)	No. of lung tumours per mouse	Median (range)
B16-F1	0, 0, 0, 1, 1, 1, 1, 2, 2, 3	1(0-3)	31, 36, 43, 47, 62, 70, 74, 86, 91, 97	74(31-97)
B16-B1	0, 0, 1, 1, 1, 2, 2, 2, 2	1(0-2)	32, 34, 72, 85, 88, 90, 93, 95, 97	85(32-95)
B16-B5	0, 1, 1, 3, 4, 5, 6, 10, 11	4(0-11)	0, 0, 0, 0, 1, 6, 6, 9, 9	1(0-9)
B16-B10n	3, 5, 7, 9, 15, 20, 21, 24, 28	9(3-28)	0, 0, 0, 0, 0, 0, 0, 0, 1	0(0-1)

Single cell suspensions of viable B16 melanoma cells were injected into the tail veins of syngeneic C57BL/6 mice, and pigmented melanoma colonies were counted after 3-4 weeks (see text).

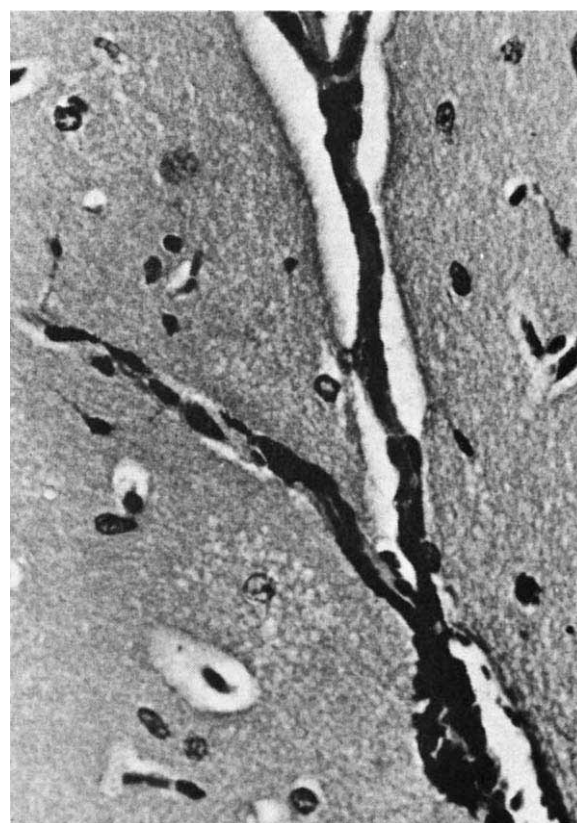


Fig. 1 Histology section of mouse brain containing B16-B10n tumours. The section is a sagittal cut through the forebrain, exposing the cerebral cortex (CC) and olfactory bulb (OB) in the region of the median sagittal rhinal fissure (RF). The B16-B10n melanoma cells (M) appear to be extending along a vascular pathway into the cerebrum. (Haematoxylin-eosin stain, x600.)

To select for brain colonising variants of B16 melanoma lines, cells were grown *in vitro* in Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with 10% foetal bovine serum, nonessential amino acids and antibiotics¹⁷. Subconfluent, low passage cells were collected, using Dulbecco's phosphate-buffered saline (PBS) containing 2 mM EDTA, into single-cell suspensions which were carefully washed twice in DMEM without serum and immediately injected into groups of syngeneic C57BL/6 mice at room temperature. For the first four *in vivo* selections for brain colonisation we introduced single, viable B16-F1 cells into the left ventricle of the heart (0.2 ml of inoculum containing 4×10^5 cells) in order initially to avoid the lung capillary bed³⁰. The few resulting pigmented brain tumour nodules were excised and minced in DMEM containing additional antibiotics (gentamicin, 50 $\mu\text{g ml}^{-1}$ and mycostatin, 50 U ml^{-1}). Melanoma cells that grew *in vitro* from brain tumour nodules (B16-B1) were passaged twice in DMEM plus 10% foetal calf serum to eliminate normal brain antigenic material and were then collected and injected intracardially into groups of C57BL/6 mice. After seven *in vivo* selections, the melanoma cells (lines B16-B7 onwards) were introduced through the tail vein at a concentration of $2-4 \times 10^4$ cells per inoculum³⁰. Assays for organ colonisation and specificity were conducted by intravenous (tail vein) injection of 2 or 5×10^4 B16 cells; animals were autopsied and gross experimental metastases were counted with the aid of a dissecting microscope 3 or 4 weeks later, respectively.

Organs of animals injected with B16 cells were also prepared for histology after fixation in 10% buffered formalin for 3-4 weeks. Cell surface labelling experiments were conducted on subconfluent parental and brain-selected B16 lines by lactoperoxidase-catalysed radioiodination³¹. For these experiments B16 cells ($>95\%$ viable by dye exclusion) were collected as before, suspended in PBS (2×10^7 cells per ml) and labelled by the use of lactoperoxidase. ^{125}I -surface-labelled cells were incubated

Table 2 Locations of experimental metastases found after intravenous injection of variant B16 melanoma cell lines

Melanoma line	Nos of animals with tumours/total no. of animals*				
	Brain	Lung	Thoracic cavity	Ovary	Other sites
B16-F1	3/8, 4/9, 7/10	8/8, 9/9, 10/10	5/8, 6/9, 6/10	3/8, 2/9, 2/10	Adrenal (1/10)
B16-B1	4/8, 4/9, 7/9	8/8, 9/9, 9/9	6/8, 4/9, 6/9	3/8, 2/9, 3/9	Lumbar spine (1/10)
B16-B5	7/8, 8/9, 10/10	4/8, 5/9, 10/10	3/8, 0/9, 5/10	0/8, 0/9, 0/10	—
B16-B10n	10/10, 10/10, 10/10	0/10, 0/10, 1/10	0/10, 0/10, 0/10	0/10, 0/10, 1/10	—

*See legend to Table 1.

for 30 min at 4 °C in 0.5% Nonidet P-40 in PBS to disrupt plasma membranes. Cell nuclei were removed by centrifugation at 4 °C, and the Nonidet P-40 supernatants were solubilised completely by boiling for 5 min in 2% SDS containing 2% 2-mercaptoethanol. Aliquots were applied to 7.5–10% linear gradient polyacrylamide slab gels³². Electrophoresis was performed, and autoradiograms were prepared from the dried gels and then scanned with a densitometer (E-C Apparatus).

Sequential *in vivo* selection for brain-preferring B16 melanoma variant lines yielded increasingly more brain-specific cell lines with selection (Table 1). Whereas B16-F1 (selected once in lung) yielded a large number of pulmonary tumours, some extrapulmonary tumours and a few brain tumours, brain-selected B16-B5 and B16-B10n formed respectively larger numbers of brain lesions and few (B16-B5) to almost no lung tumours or lesions at other sites (B16-B10n) (Tables 1 and 2). In these assays B16 melanoma cells were administered intravenously through the tail vein, so that they had to pass through pulmonary capillaries and recirculate to reach the brain. In other assays where B16 lines were administered through the left ventricle of the heart even larger differences were found between numbers of brain tumour colonies of B16-F1 and the selected line B16-B8³⁰. When the gross locations of brain tumours were assessed at selection B7, two zones of brain colonisation were found. One included the meninges of the dorsal cerebrum, and the other was restricted to the forebrain near the olfactory bulb. The cell line obtained from the former location was called B16-B7b for its somewhat larger tumour colony size, and the cell line obtained from the latter location was called B16-B7n because a large number of animals bearing B16-B7n tumours exhibited various neurological symptoms, such as staggering gait, head tilt and poor balance and orientation. Three subsequent *in vivo* selections were performed with lines B16-B7b and B16-B7n to yield B16-B10b and B16-B10n, respectively. B16-B10b did not seem to be as organ specific as B16-B10n and formed in addition to brain lesions some visceral tumours, while B16-B10n formed almost exclusively brain tumour colonies (29/30 animals injected with B16-B10n had only

brain tumour colonies; Table 2). When brains obtained from animals displaying neurological symptoms were examined histologically, most gross B16-B10n tumours were found between the lobes of the olfactory bulb and cerebral cortex in or near the rhinal or longitudinal fissures (Fig. 1). Often these B10n tumours extended along vascular pathways penetrating deep into the brain (Fig. 1).

Cell surface differences between brain-selected and lung-selected B16 melanoma lines were found when exposed surface proteins were analysed by lactoperoxidase-catalysed iodination. Autoradiograms made after SDS-polyacrylamide gel electrophoresis of ¹²⁵I-labelled surface proteins from B16 lines revealed that the brain-selected lines had a predominance of two exposed surface proteins of molecular weight approximately 95,000 (95K component) and 100,000 (100K component) compared with the parental B16-F1 line (Fig. 2). The degree of exposure of the 95K and 100K proteins correlated with the degree of brain colonisation and preference in five out of five experiments (B16-B10n > B16-B5 > B16-B1 ~ B16-F1). Although both surface proteins incorporated more ¹²⁵I on brain-selected lines compared with the B16-F1 line, the increase in ¹²⁵I incorporation with each brain selection was greater for the 100K protein than the 95K protein (Fig. 2). Because both surface proteins were labelled on the B16-F line, this indicates possible differences in display or quantity of these surface molecules on the brain-colonising B16-B lines.

The differences in cell surface labelling seen on the brain-selected B16 lines were not seen with lung-selected B16-F melanoma lines. When B16-F1 and B16-F10 (selected 10 times for lung colonisation²⁸) were compared, the 95K and 100K surface proteins were labelled with ¹²⁵I to a similar extent³³. We have found that changes in the exposure of surface proteins of ovary-selected B16-O lines correlating with the ability to colonise ovary are distinct from the changes in the surface proteins of the B16-B or B16-F lines (K.W.B. and G.L.N., to be published).

It has been suggested from cloning and fluctuation assays that highly malignant cell types with unique biological and surface properties may pre-exist in the unselected tumour cell population. Fidler and Kripke³⁴ obtained clones of B16-F1 that differed widely in their abilities to form pulmonary tumour colonies in syngeneic mice. Similar cloning experiments with a malignant sarcoma tumour line also yielded clones that possessed differing lung colonisation potential³⁵. Such experiments and our studies on selection for organ preference may yield variant tumour cell lines with distinct surface modifications that determine the extent and location of experimental metastases. Tumour cell lines that colonise the brain specifically should be especially useful in the design of therapeutic protocols for brain malignancies.

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After our manuscript had been submitted for publication, Tao and Burger³⁶ reported *in vitro* selection of nonmetastatic B16 variants by using resistance to wheat germ agglutinin cytotoxicity.

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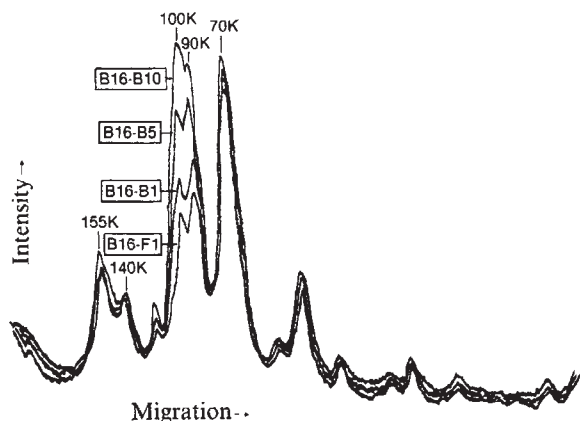


Fig. 2 Lactoperoxidase-catalysed ¹²⁵I-surface labelling of B16 lines selected for brain colonisation. The results are expressed as densitometric scans of film which were overlaid on SDS-polyacrylamide slab gels loaded with equivalent Nonidet P-40 extracts of ¹²⁵I-surface-labelled B16-F1, B16-B1, B16-B5 and B16-B10n lines, respectively. The distinctive differences in exposure of 100,000 and 95,000 molecular weight components on the brain-selected B16 lines.

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Incorporation of murine MHC antigens into liposomes and their effect in the secondary mixed lymphocyte reaction

THE major histocompatibility complex (MHC) controls the mixed lymphocyte reaction (MLR) by a dual mechanism involving both the *I*-region and the *K/D*-loci products (for a review, see ref. 1). Most information about the MLR has so far been obtained by genetic studies using congenic resistant mouse strains that differ only at defined MHC loci. To dissect the events of the MLR further, it would be advantageous to use purified MHC antigens to gain control not only over the genetical but also the physico-chemical nature of the stimulating antigens. We describe here how in a first attempt to use highly purified MHC antigens as stimulatory agents in the MLR, we have succeeded in anchoring the antigens to liposomes in such a way that they give rise to a good response in the secondary MLR.

Liposomes containing highly purified H-2 K/D and Ia antigens were prepared as described in Fig. 1. Whereas liposomes made in the absence of protein had a rather uniform size and emerged at a K_{av} of about 0.4 (Fig. 1b), the protein-containing liposomes displayed a more heterogeneous elution behaviour (Fig. 1a). Some material always appeared in the void fraction of the column when protein-containing liposomes were analysed. The amount of such aggregated material was dependent on the lipid-to-protein ratio employed. At high ratios, the liposomes emerged from the column with a distribution similar to that of the protein-free liposomes, whereas at low ratios the liposome peak was skewed towards the void fractions which contained significant amounts of material. In the analyses to be described below, only liposomes eluted in the K_{av} range 0.35-0.45 were used. This fraction comprised a mixture of free and protein-containing liposomes.

To ascertain that the protein-containing liposomes exposed the proteins in a way similar to the cell membrane,

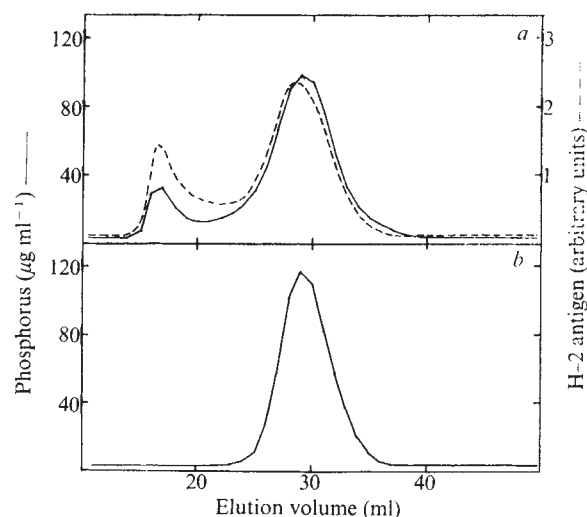


Fig. 1 Gel chromatography of MHC-antigen-containing (a) and protein-free (b) liposomes on a column (60 × 1 cm) of Sepharose 4B equilibrated with 0.01 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl. H-2 K/D antigens and Ia antigens were isolated from splenocytes. Three separate preparations were made from spleens derived from B10, B10.BR, and B10.S(7R) spleens. Crude membrane fractions were solubilised with sodium deoxycholate³. Soluble glycoproteins were isolated by affinity chromatography on a column of Sepharose 4B containing *Lens culinaris* haemagglutinin². Bound molecules were desorbed with the competitive ligand α -methylmannoside. Subsequent purification was achieved by fractionation steps involving gel chromatography on Sephadex G-200 in a buffer containing deoxycholate, ion-exchange chromatography and Sepharose 6B gel chromatography in buffers containing Tween-80, and immunosorbent chromatography (ref. 9 and B.C. and P.A.P., unpublished results). On SDS-polyacrylamide gel electrophoresis, the highly purified MHC-antigen preparations gave rise to four distinct protein bands with the apparent molecular weights 12,000, 27,000, 35,000, and 46,000, respectively. The relative amounts of the stain Coomassie brilliant blue bound to the four polypeptide chains were approximately 1/1.5/1/5.5, as revealed by measurements of the absorbance at 570 nm in a Gilford Linear transport scanner. ¹²⁵I-Labeling¹⁰ of the MHC-antigen preparations followed by SDS-polyacrylamide gel electrophoresis revealed the same four components as visualised by protein staining techniques. Thus, by these criteria no protein in addition to the four polypeptide chains was evident. Immunosorbent columns containing either covalently bound rabbit antibodies against β_2 -microglobulin¹¹ or against highly purified papain-solubilised H-2 antigens^{12,13} completely removed both the 12,000 and the 46,000 MW polypeptide chains from the MHC antigen preparations. This strongly suggested that the smallest and the largest of the four polypeptide chains were β_2 -microglobulin and the heavy H-2 K/D antigen chain. Indirect immunoprecipitation¹⁴ with use of a rabbit anti-HLA-D antigen serum¹⁵, which crossreacts with murine Ia antigens¹⁶, and SDS-polyacrylamide gel electrophoresis, demonstrated that only the 27,000 and the 35,000 MW polypeptide chains in the MHC-antigen preparations reacted with this antiserum. Crosslinking experiments¹⁶ showed that the 27,000 and the 35,000 MW chains were held together in non-denaturing conditions. These data are fully consistent with the view that the 27,000 and the 35,000 MW chains were Ia antigen subunits¹⁷. The highly purified antigens in 0.01 M Tris-HCl buffer, pH 7.4, containing 0.05 M NaCl and 0.05 M cholate were mixed with egg lecithin (Sigma) and liposomes were formed as described¹⁸. The lecithin concentration was invariably 20 mg ml⁻¹ and the protein concentration about 0.2-1 mg ml⁻¹. The cholate was removed by gel chromatography on a Sephadex G-50 column (25 × 1 cm) equilibrated with 0.01 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl. Material eluted in the void fraction, representing free liposomes as well as liposomes containing bound protein, was subjected to Sepharose 4B gel chromatography. a Shows the elution pattern of liposomes made in the presence of B10.BR mouse spleen MHC antigens. The protein-to-lipid ratio was 1:50; b shows the pattern obtained with protein-free liposomes. Lipid phosphorus (—) was determined by a micromodification method¹⁹, and H-2 K/D antigens (---) were monitored by a radioimmunoassay¹³.

the liposomes were subjected to affinity chromatography on a *Lens culinaris* haemagglutinin column². The liposomes that passed the column were mostly free of protein, but some of the unretarded liposomes contained protein that was exclusively orientated in the inside-out (IO) direction (see below). The liposomes desorbed from the column by the elution with α -methylmannoside were largely homogeneous in the analytical ultracentrifuge, and displayed a sedimentation constant of about 4.1S. As protein-free liposomes had an apparent sedimentation constant of 2.7S, and both types of liposome exhibited Stokes' radii of about 170 Å, it can be calculated that the protein-containing liposomes contained, at the most, about 100,000 dalton of protein per liposome (B. C. and P. A. P., in preparation). This suggests that a maximum of two MHC-antigen molecules per liposome could be present, assuming that the average molecular weights of the H-2 K/D and Ia antigens are about 60,000.

To prove rigorously that only MHC antigens of one type were present in each liposome, the liposomes were subjected

to an immunosorbent column containing a rabbit anti-H-2 antigen serum. Figure 2 shows that the bound liposomes contained exclusively the 12,000 and 46,000 MW polypeptide chains, whereas the liposomes which passed the column unretarded contained nothing but the 27,000 and 35,000 MW Ia-antigen chains. As both types of molecule reacted with the relevant alloantisera (Fig. 2), these data strongly suggest that each liposome contained only one MHC-antigen molecule which had retained its antigenic properties and was orientated in the right-side out (RO) direction.

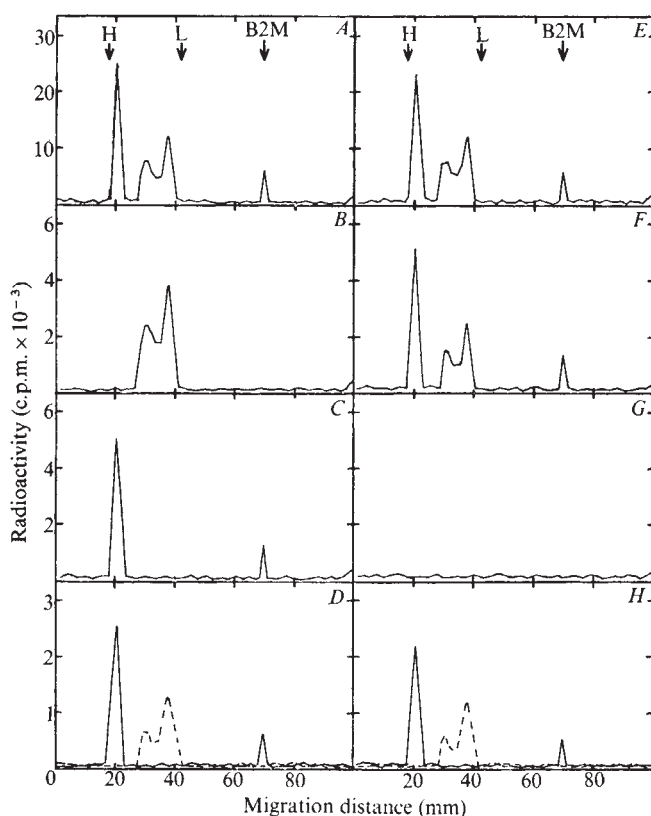


Fig. 2 SDS-polyacrylamide gel electrophoreses of ¹²⁵I-labelled B10.BR mouse spleen MHC-antigens (H-2^k) incorporated into liposomes. Liposomes bound and desorbed from a *Lens culinaris* haemagglutinin column (A–D), and liposomes which passed the column unretarded (E–H), were separately analysed. Part of each liposome fraction was directly subjected to SDS-polyacrylamide gel electrophoresis (A and E), whereas the remainder was fractionated on Sepharose 6B columns containing covalently bound rabbit anti-H-2 antigen antibodies¹³. Liposomes which passed the columns unretarded (B and F), as well as bound liposomes (C and G), were directly analysed. To examine if the MHC antigens incorporated into the liposomes had retained their alloantigenic properties, portions of the two liposome fractions obtained from the *Lens culinaris* haemagglutinin fractionation step were solubilised with 20 mM Na-deoxycholate, and proteins reactive with alloantisera directed against D^k ((ATL × B10.A)F₁ anti-B10.BR) and Ia^k ((ATH × B10)F₁ anti-ATL) were separately isolated by indirect immunoprecipitation, and subjected to SDS-polyacrylamide gel electrophoresis. D^k (—) and Ia^k (---) antigens reactive with the alloantisera were present in both liposome fractions (D and H). All samples were run on 12% polyacrylamide gels⁴. The arrows denote heavy (H) and light (L) immunoglobulin chains and β_2 -microglobulin (B2M).

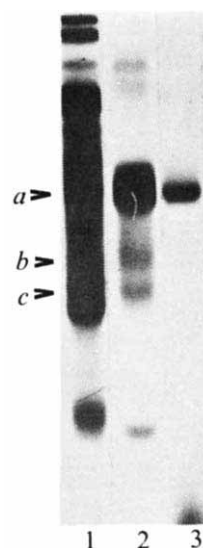


Fig. 3 SDS-Polyacrylamide gel electrophoresis of proteins not incorporated into liposomes (1), RO-liposomes (2) and IO-liposomes (3). The migration positions of K/D antigens and the two Ia-antigen subunits are indicated by a, b and c, respectively. β_2 -Microglobulin does not show up as it migrated with the dye front. Because of the large lipid-to-protein ratio, only limited amounts of protein from the IO-liposome fraction could be subjected to the SDS-polyacrylamide gels to avoid lipids interfering with the separation. On the original gels the two Ia-antigen subunit bands and the two high-molecular weight contaminants were clearly discernible. Solubilised crude membrane proteins to be inserted into the liposomes were enriched for H-2 K/D and Ia antigens by lectin affinity chromatography and gel chromatography on a column of Sephadex G-200. Binding of the MHC-antigens to the liposomes and their fractionation were achieved as described in Fig. 1.

The liposomes that passed the *Lens culinaris* haemagglutinin column unretarded were composed of a mixture of free and protein-containing liposomes, as shown by analytical ultracentrifugation. The major portion of the liposomes had a sedimentation constant of 2.7S, although a small but significant portion sedimented as 4.1S material. We failed to observe binding of any of these liposomes to columns containing antibodies against β_2 -microglobulin and H-2 antigens, although all the four MHC-antigen polypeptide chains were present in this liposome fraction (Fig. 2). These data seem to suggest that the analysed fraction of liposomes contained MHC antigens exclusively orientated in the IO direction.

When isolating MHC antigens from highly purified membrane preparations, it has been observed that only a few contaminating proteins of sizes similar to the MHC antigens are present in significant amounts³. However, if the starting material consists of crude membrane preparations, like a 100,000g pellet, several proteins that do not bind detergent are difficult to separate from the MHC antigens by conventional fractionation techniques. Attempts were therefore made to incorporate MHC antigens into liposomes from crude fractions containing several of these contaminating proteins (see Fig. 3 legend). As it is probable that only detergent-binding proteins are incorporated into

the liposomes, a considerable purification of the MHC antigens was achieved during the liposome-preparation procedure. Figure 3 shows that RO-liposomes on SDS-polyacrylamide gel electrophoresis⁴ contained mostly Ia and H-2 K/D antigens, although small amounts of a few high-molecular weight proteins were also visible. The gel profile for the IO-liposomes was similar to that for the RO-liposomes, but quite distinct from the electrophoretic pattern for the proteins not incorporated into the liposomes. The latter proteins, which are probably not membrane proteins, were eluted late from the Sepharose 4B-column. Thus, the present results suggest that liposome incorporation of MHC antigens may serve as an excellent purification step and may obviate the need to use highly purified plasma membranes for the isolation of the antigens.

It is well established that K/D antigens and Ia antigens, respectively, stimulate different T-lymphocyte populations⁵⁻⁷. To analyse the stimulatory capacity of the purified K/D antigens and the Ia antigens separately, the primary MLR

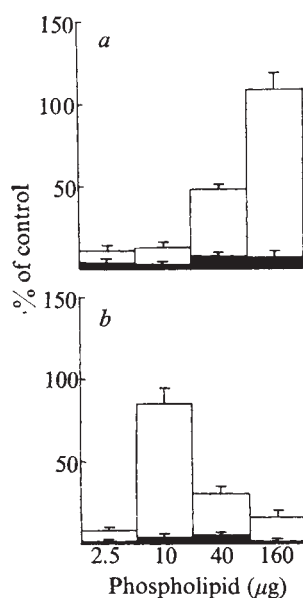


Fig. 4 Stimulatory effect of the MHC-antigen-containing liposomes on the proliferative response in the secondary MLR. Primary MLR was performed by culturing 2×10^6 mitomycin-C treated ATL (*skkd*) stimulator spleen cells per ml with 2×10^6 responder cells per ml. The responder cells were obtained from lymph nodes of B10 (*bbbb*) mice and a T-lymphocyte enriched fraction was used²⁰. The cells were maintained in 10 ml of Dulbecco's modified Eagle's medium (DMEM) containing 5×10^{-5} M 2-mercaptoethanol, 50 $\mu\text{g ml}^{-1}$ neomycin, and 10% foetal calf serum in upright Falcon tissue culture flasks in 5% CO_2 and humidified air. After 5 d of culture, blast cells were isolated by foetal calf serum gradient separation²¹, and aliquots of 4×10^4 cells were placed in 100 μl of DMEM in round-bottom microtitre plates. After 24 h incubation to let the primary proliferative response decline, 20 μl of liposomes or fresh stimulator cells (2×10^5 cells per well) were added. The secondary proliferative MLR response was measured after an additional 2 d by the incorporation of ^3H -thymidine (1 μCi per well) during the last 8 h of culture. In **A** the liposomes contained MHC antigens isolated from B10.S(7R) (*sssd*) splenocytes, and in **B** the MHC antigens were derived from B10.BR (*kkkk*) mice. The liposomes (the initial phospholipid amount was 160 μg and the protein amount was 3 μg) were added in various dilutions, as indicated in the figure. The open symbols denote the response obtained with RO-liposomes and the solid symbols denote the effect of the IO-liposome fraction. The bars represent the standard deviation of quadruplicate experiments. The data are expressed as % of the net response (allogeneic response – syngeneic response) obtained after stimulating the primed cells with splenocytes of the relevant haplotype. The liposome-mediated stimulation was obtained as the response measured after stimulation with allogeneic antigens minus the response obtained with an equivalent amount of liposomes containing the same quantity of syngeneic MHC antigens from B.10 mice.

was performed over a K,I,D difference. The primed responder cells⁸ were then incubated with liposomes containing MHC antigens identical to those of the primary stimulator cells either with regard to the K/D antigens (B10.S(7R)) or to the Ia antigens (B10.BR). RO- as well as IO-liposome fractions were used, and liposomes containing MHC-antigens from B10 mice served as the control.

At high concentrations, liposomes without protein or with syngeneic MHC antigens slightly depressed the ^3H -thymidine background incorporation, so all experiments had to be performed with carefully controlled quantities of liposomes. Figure 4 shows that RO-liposomes containing relevant K/D antigens promoted a good secondary response that was concentration dependent. As expected, IO-liposomes failed to cause proliferation of the responder cells. RO-liposomes containing relevant Ia antigens also caused a significant proliferate response. Also, the Ia antigens, in contrast to the K/D antigens, were less effective at high than at medium concentrations in stimulating the responder cells (Fig. 4). This observation was consistent in all the experiments performed, and suggests that the dose-dependent stimulation is different for the Ia antigens compared to the K/D antigens. Although the quantitative aspects of the liposome-mediated stimulation are still unclear, it is notable that the liposome fractions contained severalfold more of the K/D than of the Ia antigens.

Because of their amphipathic nature, the highly purified MHC antigens have to be kept in detergent solutions to remain soluble, thus precluding their use in cell assays, as detergents usually solubilise cell membranes. However, the present study shows that liposome-bound MHC antigens, orientated in the RO direction, may be useful tools in exploring the cellular effects of the antigens. It is anticipated that variations of the lipid composition, the size, the charge, the protein content and the geometrical configuration of the inserted proteins in the liposomes may be of importance in tailoring the best reagents for such studies.

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Assignment of a type I collagen structural gene to human chromosome 7

COLLAGEN is the major extracellular protein of the body, and forms the fibres which impart tensile strength to all of its structural elements, including skin, bone, tendon and cartilage. It occurs as a family of molecules with closely related structures but distinctive tissue distributions¹. Four types have been described with varying degrees of precision; the most abundant and best studied (type I) is a heteropolymer of three polypeptides, two identical ($\alpha_1(I)$), and a third ($\alpha_2(I)$) which differs slightly in primary structure. Types II–IV are homopolymers of the general formula ($\alpha_1(x)$)₃, where the designation α_1 reflects the close sequence homologies shared with $\alpha_1(I)$, although this relationship is only tentative in the case of $\alpha_1(IV)$. These collagens are probably the products of at least five non-allelic structural genes, and knowledge of their linkage would be useful in view of their possible origin by duplication and divergence from an ancestral collagen gene. Quantitative variations in the expression of these genes are important in normal tissue development, and defective expression has been implied in two inherited disorders, Ehlers-Danlos IV² and osteogenesis imperfecta³. The chromosomal assignment of these structural genes is a first step towards discovering their linkage relationship and perhaps towards understanding more about their differential expression. Because collagen is readily synthesised by cells *in vitro*, the problem of chromosome assignments may be investigated by the techniques of somatic cell genetics. When mouse and human

cells are fused, random loss of human chromosomes occur, while the full set of mouse chromosomes is usually retained. Assignment of a gene to the chromosome carrying it can be achieved by examining sets of hybrids for expression of the human gene product and ascertaining the segregation of this phenotype with a particular human chromosome. We have examined a series of human–mouse hybrid clones for the production of collagen and report here the consistent segregation of human collagen with human chromosome 7.

To distinguish human from mouse collagens, species-specific antibodies were prepared against type I collagen by inoculation of sheep with type I collagen prepared from acetic acid extracts of human foetal skin or mouse tail tendon⁴. Animals received fortnightly subdermal injections of 5 mg of collagen suspended in saline and Freund's complete adjuvant⁵. The fortnightly bleeds were tested by double

Table 1 Inhibition of agglutination by medium from cultured fibroblasts and by collagen

Inhibitor	Average titre decrease ($-\log_2$)	
	Human collagen	Mouse collagen
DUV (Primary human fibroblast)	5	0
KEN (Primary human fibroblast)	3.5	0
NOC (Primary human fibroblast)	5	0
MRIL (Primary human fibroblast)	5	0
LNSV (Transformed human fibroblast)	4	0
L929 (Mouse L cell)	0	3
A9 (Mouse L cell)	0	3
1R (Mouse L cell)	0	2.5
C1 1D (Mouse L cell)	0	1
1.1FB2 (Primary mouse fibroblast)	0	5.5
Human collagen		
$\alpha_1(I)^*$	5	0
$\alpha_1(III)^\dagger$	2	0
$\alpha_2(I)^*$	7.5	0
Mouse collagen		
$\alpha_1(I)^*$	0	2
$\alpha_2(I)^*$	0	3

50 μ l of heat-denatured medium or collagen solution (10 μ g ml⁻¹) were incubated in agglutination plates with 50 μ l of serially diluted antibody. To test for human collagen antigens this antibody was purified by two-step immunoabsorption (see text) from sheep anti-human collagen antiserum. As an additional precaution excess mouse collagen was added to 100 μ g ml⁻¹. After incubation for 30 min at room temperature, a 1% suspension of sheep erythrocytes coated with human collagen was added and the plates read after settling overnight, again at room temperature. Mouse collagen antigens were tested in an exactly analogous way using purified anti-mouse collagen antibody blocked with excess human collagen and incubated with mouse collagen-coated sheep erythrocytes. Inhibition of agglutination is expressed as ($-\log_2$) titre decrease compared to the end-point achieved with either fresh medium or solvent as appropriate. In different experiments these reference end-points were 1/640 for mouse collagen and either 1/1,280 or 1/2,560 for human collagen.

Cells were grown to confluence (approximately 2×10^6 cells) in RPMI 1640 containing 10% foetal calf serum and 0.35 mM ascorbate. Before testing, medium was denatured by heating at 60 °C for 30 min.

*Prepared from acid-soluble collagen by CM-cellulose chromatography⁴.

†Prepared from pepsin-soluble human foetal skin.



Fig. 1 Metaphase of clone 21 with G-11 banding¹⁰ showing three copies of human chromosome 7 (arrows).

immunodiffusion against heat denatured collagen, and the animals were bled out when the titre reached a plateau. Precipitin arcs formed in this reaction were susceptible to *in situ* digestion by purified bacterial collagenase.

The possibility that the reagents identify antigenic sites which result from the activity of modifying enzymes must be excluded if they are to be used subsequently to detect the products of the structural genes. Pre-treatment of collagen with periodate to remove covalently bound sugars did not affect the formation of precipitin arcs, implying that the principal antigenic sites on the collagen molecule were not a product of post-translational glycosylation. Two such hyperimmune antisera cross-reacted to some extent with mouse and human collagen. These were purified by passage through collagen–CNBr–Sephadex conjugates of opposite specificity before absorption to and elution from conjugates of collagen of the same specificity. These fractions, and the crude antisera from which they had been prepared, were examined by haemagglutination using sheep erythrocytes coated with heat-denatured collagens⁶. The titres of the anti-human and anti-mouse antisera were 1/10,000 and 1/2,500, respectively. After purification these were 1/2,500 and 1/640, with cross-reacting antibody titres of less than 1/20.

Table 2 Human and mouse collagen production by somatic cell hybrids*

Hybrid	Parents		Collagen		Chromosome																						
	H	M	H	M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
DUR 4 (7)	1°FIB	×IR	0	4.5			+		+					+	+	+	+	+	†		+	+		+	+	+	†
DT1-2R (8)	1°FIB	×IR	0	3			+					+		+	+		+				+	+		+	+	+	+
P2A	1°FIB	×CIID	0	2														+			+			+	+	+	+
P7A	1°FIB	×CIID	0	2			‡														+						
Transformed																											
F4/SC/13	FIB	×RAG	2	3							+		+					+								+	
MIRL 110	1°FIB	×IR	1	3							+	§		+				+									
MIRL M7	1°FIB	×IR	3	4.5							+																
Clone 10	LNSV	×MAC**	4	4.5							+	¶															
Clone 21	LNSV	×MAC**	3	5							+																

*Assayed as in Table 1.

†Contains an X/15 translocation: 46, X, t(X; 15) (p11, q11).

‡Contains a 3p deletion.

§Contains an X/7 and 7/X: 46, Y, t(X; 7) (q22-24, q22) and an X/7 with 7p deletion at 7p (13-15), in addition to an intact 7.

||Contains an X/7 with 7p deletion, as above, in addition to an intact 7.

¶Contains an unidentified human/mouse translocation, in addition to an intact 7.

**Peritoneal macrophages. These hybrids lack normal macrophage markers (S. Gordon, personal communication) and medium from cultured mouse macrophages does not normally contain measurable mouse collagen by this test.

Fibroblasts grown *in vitro* synthesise collagen which is secreted into the medium as the soluble precursor, procollagen. Media from confluent cultures of human and mouse fibroblasts were examined for their ability to inhibit the agglutination of collagen-coated sheep erythrocytes by these antibodies (Table 1). In all cases a high degree of inhibition was achieved in only one of the two tests, and this result was always consistent with the species of cultured cells from which they were derived.

Collagen (5 µg in 50 µl) from the correct species completely inhibited agglutination by undiluted antibodies. When separated chains of human collagen were tested, the ability of α₂(I) to inhibit agglutination was more than 10 times greater than that of α₁(I) and 30 times greater than α₁(III). The sensitivity of this assay (1/128 dilution of some culture media still gave detectable inhibition) enabled us to examine media from several cell lines without concentration or any other special treatment.

A selection of interspecific human-mouse hybrid clones was examined for both human and mouse collagen production (Table 2). Most of these hybrids were from human fibroblast-mouse L cell fusions, and both parental cells produced collagen. The ability to synthesise mouse collagen was retained in all hybrids examined. Four of the hybrids did not secrete detectable amounts of human collagen, and examination of their karyotypes eliminated as candidates chromosomes 3,5,8,10,11,12,13,14,17,18,20,21,22 and X. However, two hybrids in the first set examined, F4/SC/13 and MIRL 110, produced detectable levels of both mouse and human collagen, and comparison of their chromosomes with those of the non-producers indicated that this ability segregated with human chromosome 7. Subsequently, three hybrids, reported to have greatly reduced numbers of human chromosomes, but retaining chromosome 7, were found to synthesise both mouse and human collagen. Karyotypic analysis carried out at the same passage confirmed chromosome 7 as the common denominator. Clone 21 contained only this human chromosome (Fig. 1).

These experiments demonstrated that hybrid cells can synthesise and secrete human collagen when 7 is the only human chromosome present. The cells must, therefore, contain the necessary apparatus for processing the human gene product sufficiently to permit its detection in the medium. Unless the necessary components, notably the post-translational modifying enzymes, are also coded for by genes on chromosome 7, it follows that mouse enzymes must be capable of performing these operations. Nevertheless, the consistent segregation of human collagen production with chromosome 7 can only mean that at least one

important collagen structural gene is located on this chromosome.

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Molecular mechanism of physiological fibrinolysis

THE proteolytic enzyme system in blood that is predominantly responsible for removal of fibrin deposits, is called the fibrinolytic system. This system consists of three main components: the proenzyme plasminogen, which can be activated by limited proteolysis to the proteolytic enzyme plasmin; plasminogen activators, the most important of which probably originates in the endothelial cells; and inhibitors, which can rapidly neutralise plasmin or interfere with the activation of plasminogen. The proteolytic enzyme plasmin has a broad specificity, which is not very different from that of trypsin. However, *in vivo* the main target of plasmin is fibrin. Three hypotheses have been put forward to explain this specificity. Alkjaersig *et al.*¹ have suggested that plasminogen is adsorbed to polymerising fibrin and converted to active enzyme by activators which diffuse into

the thrombus. Plasmin would then exert its action in an environment relatively free of inhibitors. Ambrus and Markus² have proposed that plasmin-inhibitor complexes formed in the circulation dissociate in the presence of fibrin, because plasmin has a greater affinity for fibrin than for its inhibitors. Chesterman *et al.*³ suggested that the activators bind selectively to fibrin and transform plasminogen, which diffuses into the thrombus, to plasmin. During the past few years specific interactions at the molecular level have been demonstrated between the different components of the fibrinolytic system. These findings now enable us to formulate a molecular model for the regulation of fibrinolysis *in vivo*.

In purified systems, it was found that the native form of plasminogen (with NH₂-terminal glutamic acid) has a weak affinity for fibrin, whereas partially degraded plasminogen (with NH₂-terminal lysine) formed by autocatalytic digestion has a stronger affinity⁴. It was later demonstrated that this interaction is mediated by way of structures in the plasminogen molecule; these are known as lysine-binding sites, as they are also responsible for its interaction with certain amino acids having antifibrinolytic action *in vivo*, such as lysine or 6-aminohexanoic acid⁵. Accordingly, the fibrin-plasminogen interaction is abolished by these amino acids^{4,5}.

We have recently investigated the adsorption of plasminogen to fibrin in a whole plasma system, by clotting the system after adding a trace amount of radiolabelled plasminogen. Approximately 4% of native and 8% of partially degraded plasminogen was specifically adsorbed to the fibrin⁶.

Kinetic and structural work both indicate that the plasminogen molecule contains at least two lysine-binding sites^{5,7,8}. The higher affinity of the partially degraded form of plasminogen for fibrin might be explained by the fact that both its lysine-binding sites are available, whereas in the native plasminogen one of them is occupied by an interaction with a specific site in the NH₂-terminal part of the molecule⁹.

Tissue plasminogen activator obtained from pig heart has a strong affinity for fibrin¹⁰, a property which can be used for efficient purification of this protein¹¹. The human vascular plasminogen activator behaves similarly¹².

Fibrin markedly enhances the activation of plasminogen by tissue activator and human vascular activator^{11,12}. This seems to be due to the adsorption of both plasminogen and activator to the fibrin network. Other plasminogen activators, such as urokinase and streptokinase-plasminogen complex, do not have such specific affinity for fibrin, although some adsorption of these activators to the fibrin surface has been reported³.

Human plasma contains a recently discovered fast-acting and physiologically important plasmin inhibitor¹³⁻¹⁵, which we have called antiplasmin¹³. Plasmin added to, or formed in, plasma is very rapidly neutralised by antiplasmin, which is responsible for all of the fast-acting plasmin-neutralising activity of plasma. Antiplasmin and plasmin form a very stable stoichiometric 1:1 complex which is devoid of enzymatic activity^{14,16}. Antiplasmin can also bind to insolubilised plasminogen and can be specifically displaced by 6-aminohexanoic acid, a property which has been used for affinity chromatographic purification of this inhibitor^{14,16}. These findings suggest that this interaction is also mediated by way of the lysine-binding sites in the plasminogen molecule. Accordingly, it has been found in purified systems that fibrin and antiplasmin compete for the binding to plasminogen¹⁷. In plasma systems, however, the binding of plasminogen to fibrin is not significantly influenced by antiplasmin⁶.

A kinetic analysis of the reaction between plasmin and antiplasmin^{18,19} has revealed that it occurs in two steps: a

very fast reversible complex formation followed by a slower irreversible transition. The rate of the initial complex formation is the fastest so far described for protein-protein interactions¹⁹. However, in the presence of 6-aminohexanoic acid, the reaction rate is decreased by at least two orders of magnitude, indicating the importance for the reaction rate of free lysine-binding sites in the plasmin molecule. It has been further demonstrated that plasmin with a substrate molecule bound to its active site does not react with antiplasmin¹⁹. These findings imply that plasmin which is bound to fibrin and involved in its degradation, in contrast to free plasmin, is only slowly inactivated by antiplasmin.

To determine whether the initial complex between plasmin and antiplasmin (dissociation constant about 2×10^{-10} M) would dissociate in the presence of fibrin, the following simple experiment was undertaken. A mixture of fibrinogen (6 μ M), antiplasmin (1 μ M) and plasmin (0.7 μ M) was clotted by addition of thrombin, and the mixture incubated at 37 °C. No signs of clot lysis could be detected, even after 24 h, whereas in the absence of antiplasmin the lysis time was less than 1 min (unpublished results). Thus, the plasmin-antiplasmin complex does not seem to dissociate in the presence of fibrin.

On the basis of these interactions between the components of the fibrinolytic system, a molecular model for physiological fibrinolysis can be deduced, which explains the restricted action of plasmin *in vivo*. The system seems to be regulated at two levels: localised plasminogen activation at the fibrin surface; and sequestration of the formed plasmin from circulating antiplasmin. The lysine-binding sites in the plasminogen molecule play a key role in these regulations. When fibrin is formed, a small amount of plasminogen is specifically bound to it by way of its lysine-binding site(s). Plasminogen activator present in the blood or released from the vascular endothelium is adsorbed on the fibrin surface and efficiently activates the adsorbed plasminogen. The formed plasmin has its lysine-binding site(s) occupied in complex formation with fibrin and is also involved in fibrin degradation by way of its active site, and would therefore react only very slowly with antiplasmin. In contrast, plasmin which is released from digested fibrin is rapidly and irreversibly neutralised by antiplasmin in the circulation.

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Commercial ATP containing traces of vanadate alters the response of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ to external potassium

WE have reported that the puzzling inhibition of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ preparations by K^+ ions, at concentrations insufficient to displace Na^+ ions from the intracellular Na^+ -loading sites, was observed only when Sigma 'Sigma-grade' ATP was used as a substrate¹. The effect was attributed to a contaminant of the ATP, which bound to the enzyme reversibly but with a high affinity; and because 'Sigma-grade' ATP is unique in being prepared from muscle, we suggested that the inhibitor might have a physiological role. Similar conclusions were reached independently by Cantley and Josephson^{2,3}, and they and their colleagues have now identified the contaminant as orthovanadate⁴. The inhibition of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ by vanadate plus K^+ ions is of interest both for its possible physiological significance—traces of vanadate occur widely in animal tissues⁵—and for its possible use in elucidating the mechanism of the sodium pump. From both points of view it is desirable to know whether the K^+ ions act at the intracellular or extracellular surface of the membrane, and we report here experiments on resealed red cell ghosts showing that the action of K^+ ions is at the extracellular surface. In the following paper, Cantley *et al.*⁶ describe experiments with intact red cells suggesting that the vanadate ions act at the intracellular surface.

Table 1 summarises the results of four experiments in which resealed red cell ghosts containing either Sigma 'Sigma-grade' ATP or Boehringer ATP, with different concentrations of K^+ ions, were incubated at 27 °C in media containing different concentrations of K^+ . The rates of ATP hydrolysis in the presence and absence of ouabain were estimated by measuring the orthophosphate released in

45 min; preliminary experiments (data not shown) demonstrated that hydrolysis was linear with time over this period. It is clear that the rate of ouabain-sensitive hydrolysis was not affected by the changes in the intracellular K^+ concentration. When the extracellular K^+ concentration was changed, however, the rate of ATP hydrolysis was affected in a way which depended on the source of the ATP in the ghosts. With Boehringer ATP, an increase in the extracellular K^+ concentration from 5 mM to 20 or 38 mM caused a small increase in the ouabain-sensitive hydrolysis. This is the 'normal' response, and reflects the fact that a concentration of 5 mM K^+ is not quite sufficient to saturate the sodium pump in human red cells⁷. With Sigma 'Sigma-grade' ATP, an increase in the K^+ concentration from 5 mM to 38 mM caused a decrease in the rate of hydrolysis. This is characteristic of the modified response to K^+ ions which we reported in earlier experiments on a kidney $(\text{Na}^+ + \text{K}^+)\text{ATPase}$, in which the K^+ concentrations must have been the same at both membrane surfaces. The ouabain-insensitive hydrolysis of ATP was identical whether the ghosts contained Boehringer or Sigma 'Sigma-grade' ATP.

The finding that it is extracellular K^+ ions that inhibit the enzyme disposes of a suggestion made in our earlier report¹ that the effect of K^+ might be related to the inhibition by intracellular K^+ of the sodium pumps found in the low-potassium red cells of certain ungulates. It is possible that there is a connection between the action of K^+ ions in inhibiting $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ activity in the presence of vanadate and their action in promoting the hydrolysis of *p*-nitrophenyl phosphate (pNPP) by $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ preparations; both actions are extracellular and, in some conditions, the stimulation of pNPP hydrolysis seems to involve K^+ -binding sites of intermediate affinity. In experiments with an alkaline phosphatase from *Escherichia coli*, Lopez *et al.*⁸ found that hydrolysis of pNPP was competitively inhibited by both phosphate and vanadate, which suggests

Table 1 Inhibition of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ activity of resealed red cell ghosts by extracellular but not intracellular K^+ ions, when Sigma 'Sigma-grade' ATP is used as substrate

Expt	ATP	$[\text{K}]_i$ (mM)	$[\text{K}]_o$ (mM)	Ouabain-sensitive ATP hydrolysis (mmol P_i (l.cells) ⁻¹ h ⁻¹)	Statistical significance of differences
1	Boehringer	5	5	1.38 ± 0.09	None significant
		20	5	1.35 ± 0.09	
	Sigma	5	5	1.30 ± 0.05	
		20	5	1.28 ± 0.03	
2	Sigma	5	5	1.51 ± 0.04 (a)	a-b $P < 0.02$
		5	38	1.28 ± 0.05 (b)	c-d $P < 0.02$
		40	5	1.56 ± 0.08 (c)	c-a not significant
		40	38	1.17 ± 0.08 (d)	b-d not significant
3	Boehringer	5	5	1.88 ± 0.05 (a)	b-a $P < 0.05$
		5	38	2.11 ± 0.05 (b)	d-c $P < 0.05$
		40	5	1.91 ± 0.04 (c)	c-a not significant
		40	38	2.07 ± 0.05 (d)	b-c not significant
4	Boehringer	20	5	1.72 ± 0.06 (a)	b-a not significant
		20	20	1.82 ± 0.03 (b)	c-a not significant
	Sigma	20	5	1.71 ± 0.05 (c)	d-c $P < 0.05$
		20	20	1.52 ± 0.05 (d)	d-b $P < 0.005$

Red cells from freshly drawn, heparinised, human blood were washed four times with a solution containing: 150 mM NaCl, 1 mM MgCl_2 , 2 mM HEPES (Tris salt, pH 7.5, at 20 °C) and 0.1 mM EGTA. After being packed at 1,700 g for 10 min, they were lysed by being squirted into 20 volumes of an ice-cold solution containing: 6 mM ATP (Na salt adjusted to pH 7.5 with NaOH), 6 mM MgCl_2 , 2 mM HEPES (Tris salt, pH 7.5, at 20 °C) and 0.2 mM EGTA. Tonicity was restored by the addition of suitable mixtures of 2 M NaCl and KCl, and the ghosts were resealed by incubation at 37 °C for 10 min. The ghost suspensions were cooled to 0 °C and centrifuged for 5 min at 21,000 g, and the ghosts were then washed three times at 0 °C with a wash solution like that used for washing the original cells. The washed ghosts were resuspended at a haematocrit of 20% in ice-cold solutions containing: 1 mM MgCl_2 , 2 mM HEPES (Tris salt, pH 7.5, at 20 °C), 0.1 mM EGTA and Na and K at the concentrations shown in the Table. The ghosts were then incubated for 45 min at 27 °C, this temperature being chosen because our earlier experiments¹ had shown that Sigma 'Sigma-grade' ATP was more inhibitory at lower temperatures. At the end of the incubation, the tubes were transferred to an ice-bath, trichloroacetic acid was added to give a final concentration of 5% (w/v), and the inorganic phosphate formed was estimated by a modification of the method of Fiske and Subbarow. Each experiment was run in triplicate; values are means ± s.e.m.

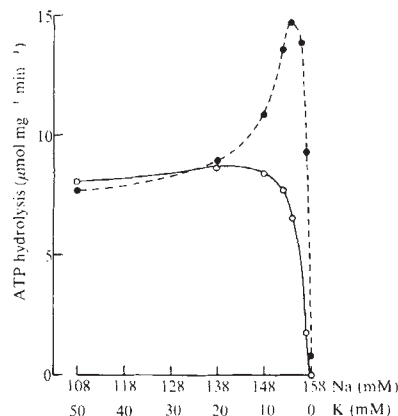


Fig. 1 An experiment showing that pretreatment of $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ with trypsin in the absence of K^+ ions, which leads to a rapid loss of about half of the activity⁹, yields an enzyme that does not show the inhibition by K^+ ions at intermediate concentrations normally seen when Sigma equine muscle ATP is used as substrate. $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ prepared from pig kidney outer medulla was incubated at 37°C for 40 min, at a concentration of 0.1 mg ml^{-1} , in 2 ml of a solution containing: 175 mM Tris/Tris Cl (pH 7.4 at 37°C), 0 or 6 μg trypsin (Sigma, twice crystallised, salt-free, from bovine pancreas; 10,000 BAEE units mg^{-1}), 30 μM EDTA, 750 μM histidine and 250 μM imidazole. Soybean trypsin inhibitor (1.7 μg of Sigma type I-S, dissolved in 0.8 ml of 25 mM imidazole (pH 7.5 at 20°C)) was then added both to the trypsin-treated and to the control enzyme suspensions, and incubation was continued for a further 5 min. Aliquots (0.025 ml) of each enzyme suspension were withdrawn and mixed with warmed 1 ml portions of suspending media containing: 3 mM Sigma 'Sigma-grade' ATP, 3 mM MgCl_2 , 30 mM histidine (pH 7.5 at 20°C), 100 μM EGTA and Na and K at the concentrations shown on the abscissa. Incubation at 37°C was continued for a further 15 min, the reaction was terminated with trichloroacetic acid (final concentration 5% w/v), and the inorganic phosphate released was estimated by a modification of the method of Fiske and Subbarow. Results similar to those shown were also obtained in experiments in which the trypsin digestion was in a medium containing: 150 mM NaCl and 25 mM imidazole (pH 7.5 at 20°C) instead of 175 mM Tris/Tris Cl. On the other hand, when half of the enzyme was inactivated by digestion with trypsin in the presence of K^+ ions—a procedure known to cause a slower monophasic loss of activity⁹—the residual activity behaved like the original enzyme in showing inhibition at intermediate K^+ concentrations when Sigma equine muscle ATP was the substrate. ●, Control enzyme; ○, trypsin-treated enzyme.

that vanadate may affect $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ by combining with a form of the enzyme that can, alternatively, combine with phosphate or pNPP. There are, however, two considerations which make this interpretation doubtful. First, in the presence of Na^+ and ATP, the hydrolysis of pNPP by $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ preparations requires only low concentrations of K^+ : in these conditions there is no evidence for an action of K^+ at binding sites of intermediate affinity. Second, $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ preparations treated with trypsin, in conditions that lead to the rapid loss of about half of the activity⁹, still show a fairly normal pattern of K^+ activation when they hydrolyse pNPP¹⁰. As shown in Fig. 1, however, such preparations show no sign of inhibition by K^+ ions at intermediate concentrations when they are hydrolysing Sigma 'Sigma-grade' ATP. Moreover, the loss of the inhibitory effect of K^+ ions seems to be associated with the loss of the inhibitory effect of vanadate, as the trypsin-treated enzyme shows similar activity with Sigma 'Sigma-grade' ATP or Boehringer ATP, at all K^+ concentrations (data not shown).

Whatever the mechanism of the vanadate effect, it is clear that the presence of traces of vanadate can not only inhibit the sodium pump, but also alter its response to changes in extracellular K^+ concentration in the physiological range. Whether the sodium pump in physiological conditions behaves as though it were under the influence of vanadate is now being investigated, but J. J. Grantham

has pointed out to us that the vanadate effect would provide a neat explanation of the puzzling inhibition of sodium transport in the proximal kidney tubule that is produced by increasing the K^+ concentration in the perfusing fluid¹¹.

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Vanadate inhibits the red cell $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ from the cytoplasmic side

THE enzyme $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ exists in the plasma membrane of animal cells and maintains a high intracellular K^+ -to- Na^+ ratio by coupling movement of these ions to the hydrolysis of ATP. This enzyme spans the plasma membrane¹ and is asymmetrically positioned with the ATP hydrolysing site on the cytoplasmic side². Cardiac glycosides inhibit the $(\text{Na}^+, \text{K}^+)\text{ATPase}$ by binding to the part of the enzyme facing the extracellular fluid³. Although there is considerable evidence that hormonal regulation of $(\text{Na}^+, \text{K}^+)\text{ATPase}$ occurs in certain tissues^{4–8}, no endogenous regulatory agents of the purified enzyme have been identified. Recently we discovered that vanadate (vanadium in the +5 oxidation state) inhibits purified $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ from several tissues at concentrations of 10^{-7} – 10^{-8} M (refs 9, 10). Vanadium has been established as a nutritional requirement in chickens and rats, and our estimates of the concentration of vanadium in muscle tissue (10^{-6} – 10^{-7} M) agree with concentrations measured in other tissues¹¹. It is known that vanadate has properties similar to those of the phosphate ion¹², and it therefore seemed likely that it might inhibit the enzyme by binding to the hydrolysis site which is on the cytoplasmic surface of the enzyme. We demonstrate here that vanadate is transported into the red cell and inhibits the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ from the cytoplasmic side.

The red cell ghost contains at least three ATPase activities: $(\text{Na}^+, \text{K}^+)$, Ca^{2+} , and Mg^{2+} ATPases. The $(\text{Na}^+, \text{K}^+)\text{ATPase}$ can be inhibited by ouabain and, as is shown in Fig. 1a, by vanadate. The affinity of vanadate for the $(\text{Na}^+, \text{K}^+)\text{ATPase}$ is 40 nM in the presence of high Mg^{2+} concentrations and 0.1 μM at physiological Mg^{2+} concentrations, in agreement with results using the kidney enzyme⁹. The effects of Na^+ , K^+ and ATP on the vanadate affinity have been discussed⁹. The activity of the $(\text{Na}^+, \text{K}^+)\text{ATPase}$ in intact red cells, estimated by the rate of rubidium uptake, is also inhibited by vanadate (Fig. 1b). The dependence of activity on the concentration of vanadate in the latter case, however, is not simple, and the apparent affinity of vanadate for the transport system in the intact cell is approximately 40 μM . This value is nearly 3 orders

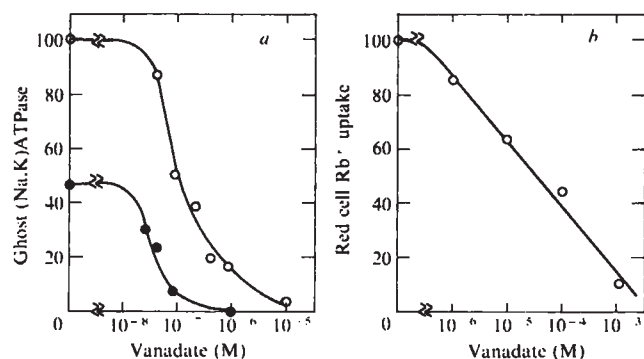


Fig. 1 *a*, The relative activity of red cell ghost (Na⁺, K⁺)ATPase as a function of Na₃VO₄ (Fischer Chemical) in the assay. The red cell ghosts were prepared by standard procedures¹⁵. The pyruvate kinase-lactate dehydrogenase-coupled assay was used in the presence of 100 mM NaCl, 25 mM KCl, 3 mM ATP (vanadate free), 1 mM [ethylenbis (oxyethylenetriol)] tetracetic acid and either 4 mM (○) or 25 mM (●) MgCl₂ as previously described⁹. Activities were measured after the product versus time curve became linear (approximately 5 min after enzyme addition). The (Na⁺, K⁺)ATPase activity was determined by subtracting the activity resistant to 100 μM ouabain (approximately 50% of the total activity). *b*, The relative rate of ouabain sensitive red cell rubidium uptake as a function of Na₃VO₄ in the red cell solution. Red blood cells were washed once with Ringers-glucose solution (145 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄, 5 mM sodium phosphate, 11 mM glucose, pH 7.4), and suspended in an equal volume of this solution. The suspension (2.5 ml per tube) was allowed to equilibrate in a water bath at 37 °C, and then various amounts of a 10 mM Na₃VO₄ solution were added to the tubes. One tube was made 0.1 mM in ouabain. After 30 min, each tube received 50 μl of Ringers-glucose solution containing 10 μCi [⁸⁶Rb] rubidium. The Aliquots (0.5 ml) were removed from the tubes at 15-min intervals, and added to 5 ml of ice cold Ringers solution. The red cells were sedimented by centrifugation, the supernatant solutions were aspirated, and 1 ml of 10% trichloroacetic acid was added to each red cell pellet. After dispersion of the pellet, the clear supernatant was removed and mixed with 5 ml of Aquasol. The radioactivity of the samples was determined by counting the β radiation in a Beckman liquid scintillation spectrometer. The incorporation of [⁸⁶Rb] rubidium into the red blood cells was linear for at least 2 h. The fraction of the rubidium incorporation which was ouabain-inhibitable was determined, and this fraction was used to calculate the relative rate of rubidium uptake as a function of the concentration of vanadate.

of magnitude larger than that obtained with purified membranes.

The equilibration of vanadate across the red cell membrane is catalysed by the anion exchange system, as is shown by the experiments in Figs 2*a* and *b*. The disappearance of ⁵¹V from the extracellular solution is described by a double exponential process (Fig. 2*a*). The initial phase ($t_{1/2} = 4$ min) is complete within 15–30 min, at which time intracellular and extracellular ⁵¹V concentrations are equal. During the slower phase ($t_{1/2} = 90$ min) the ⁵¹V continues to be concentrated to a final gradient of 500 μM_{in}/40 μM_{out}. Washing the cells with 2.5 mM noradrenaline (which forms a complex with vanadate; L.C.C., Cantley and Josephson, in preparation) does not remove the ⁵¹V, making it unlikely that it is complexing with lipids or proteins on the outside of the cell. Dinitrostilbene disulphonate, an inhibitor of anion exchange¹³, prevents the initial phase of ⁵¹V uptake (Fig. 2*b*). The (Na⁺, K⁺)ATPase activity, as judged by rubidium uptake rates, was monitored during vanadate uptake (Fig. 2*c*). The activity fell to 20% within 6 min after vanadate addition and remained at this level for more than 3 h. The onset of this inhibition was slowed by dinitrostilbene disulphonate, suggesting that vanadate uptake is essential for (Na⁺, K⁺)ATPase inhibition. Thus, three conclusions are indicated by the results in Fig. 2: vanadate equilibration across the red cell membrane is rapid ($t_{1/2} = 4$ min) and is catalysed by the anion-exchange system; vanadate inhibits the (Na⁺, K⁺)ATPase from the cytoplasmic side;

⁵¹V is accumulated in the cytoplasm of the red cell by a relatively slow process ($t_{1/2} = 90$ min).

Further evidence that vanadate enters the cells by the same anion-exchange system as phosphate is presented in Fig. 3. The affinity of vanadate for this system, as judged by the inhibition of phosphate transport, is approximately 40 mM compared to a phosphate affinity of 80 mM. However, the rate of vanadate equilibration ($t_{1/2} = 4$ min at 250 μM) is much faster than phosphate equilibration ($t_{1/2} = 1$ h at 5 mM) in the same conditions.

In view of the evidence that vanadate enters the red blood cells readily, it was puzzling that the apparent K_M for inhibition of the (Na⁺, K⁺)ATPase in the intact cell is so much greater than that for the isolated membranes. The data in Table 1 suggest that the vanadate ion alters after it enters the red blood cell, so that the activity of vanadate inside the cell is much smaller than the concentration of the various species of vanadate ion. In fact, only a small fraction of the intracellular

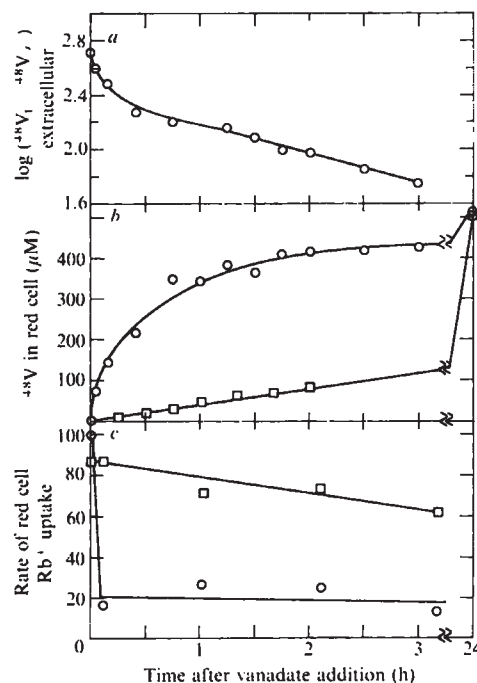


Fig. 2 *a*, A semilog plot of the time-dependent equilibration of ⁵¹V across the red cell plasma membrane. ⁵¹V_o is the extracellular vanadium concentration (μM) at time *t* and ⁵¹V_∞ is the extracellular vanadium concentration after 24 h (40 μM). Red cells were suspended in an equal volume of Ringers as described in Fig. 1*b*. At *t* = 0, the solution was made 250 μM in [⁵¹V]—Na₃VO₄. [The [⁵¹V] Na₃VO₄ was prepared by diluting [⁵¹V] VOCl₃ (in 1 N HCl; Amersham) into Na₃VO₄, adjusting the pH to 8.0 with NaOH and allowing to stand at 37 °C for at least 3 h]. Periodically, 0.5 ml of cells were centrifuged for 1 min in a Beckman Microfuge. The supernatant was removed and 200 μl were counted for ⁵¹V in a γ-counter. *b*, The pellet from the experiment in *a* (○) was counted for ⁵¹V after a single wash in 0.5 ml of cold (4 °C) Ringers. In a parallel experiment (□) the cell solution contained 580 μM of dinitrostilbene disulphonate, an inhibitor of the anion exchange system. *c*, The initial rate of rubidium uptake into the red cell at various times after addition of 250 μM Na₃VO₄. Red blood cells were prepared as described in the legend to Fig. 1*b*. At time zero, one tube (○) (10 ml of suspension) was made 250 μM in Na₃VO₄, another tube (□) (10 ml of suspension) was made 250 μM Na₃VO₄ and 580 μM dinitrostilbene disulphonate, and a third tube (Δ) (20 ml of suspension) contained only the suspension. At various times, 2 ml of suspension were taken from each container and placed in a tube with 50 μl of the Ringer solution containing 5 μCi of [⁸⁶Rb] rubidium. An additional 2-ml aliquot from the third container was put in a tube with 50 μl of the [⁸⁶Rb] rubidium solution and 20 μl of a 10 mM ouabain solution. The rubidium uptake was determined as described previously, and the relative rate of ouabain-dependent rubidium uptake estimated.

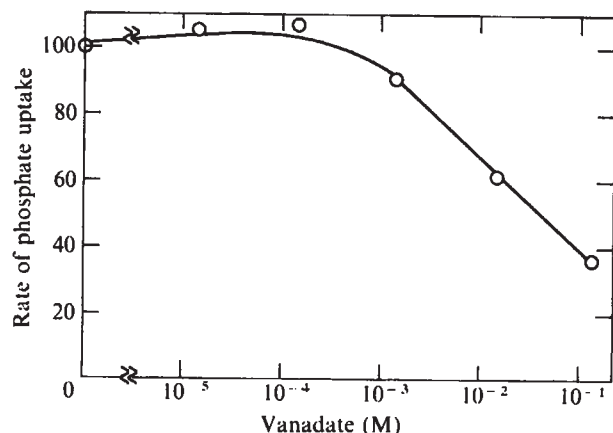


Fig. 3 The relative initial rate of $[^{32}\text{P}]$ -phosphate uptake into intact red cells as a function of Na_3VO_4 concentration in the cell solution. The cells were suspended in Ringers solution at 37°C as in all previous experiments except at a concentration of 10^{-5}M cells (v/v), in order to slow down the loss of vanadate from the extracellular solution. The vanadate was added approximately 15 s before the $[^{32}\text{P}]$ (a negligible amount of $[^{32}\text{P}] \text{H}_3\text{PO}_4$ was added to the Ringers solution containing 5 mM phosphate at time 0). The amount of ^{32}P taken up was measured at 3–4-min intervals by the procedure used in Fig. 1b. The rate of uptake was linear for the first 10–15 min for all experiments.

vanadate will pass through a membrane filter, indicating that most of the vanadate is bound to either protein (haemoglobin) or other non-filtrable molecules. This conversion requires the intact cell, because incubation of vanadate with red cell ghosts and pure haemoglobin does not result in any change in the activity of vanadate; we do not know the nature of this conversion.

Taken together, these results indicate that vanadate is effective as an inhibitor only when it interacts with the cytoplasmic surface of the $(\text{Na}^+, \text{K}^+)\text{ATPase}$. As is suggested by the work of Lienhard *et al.*¹², vanadate is probably binding to a site on the enzyme which is specific for phosphate. In the preceding paper¹⁶, Beauge and Glynn show that a potassium site on the extracellular surface of the red cell controls vanadate inhibition of $(\text{Na}^+, \text{K}^+)\text{ATPase}$ activity.

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Table 1 Distribution of ^{45}V following incubation of red cells with $[^{45}\text{V}]-\text{Na}_3\text{VO}_4^*$

	Time after addition of ^{45}V		
	0	1 h	2.5 h
Extracellular concentration (mM)	0.80	0.04	0.03
Total concentration in red cell (mM)	0	1.14	1.16
Concentration in soluble fraction from lysed red cell (mM)	0	0.91	0.91
Concentration in filtrable fraction from lysed red cell (mM)	0	0.06	0.04
Concentration bound to soluble red cell proteins (mM)	0	0.85	0.87

*The procedure for determining the extracellular and the total red cell ^{45}V concentrations was the same as in Fig. 2a and b except that $400 \mu\text{M}$ $[^{45}\text{V}]-\text{Na}_3\text{VO}_4$ was added to the 50% cell solution at zero time. The soluble fraction of ^{45}V was determined by lysing the washed cells (0.25 ml pellet) with 1 ml of distilled water, precipitating the membranes by a 5-min centrifugation in a Beckman Microfuge, and counting 200 μl of the supernatant for ^{45}V . The filtrable and protein-bound fractions were determined by forcing a portion (10 μl) of the remaining supernatant through an Amicon XM-50 membrane (which excludes proteins with molecular weights $> 50,000$) as previously described¹¹ (filtration time 10 min). The concentrations expressed were corrected for dilution during lysis so that they could be readily compared with the total concentration in the red cell.

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Inorganic polyphosphate involved in the symbiosis between chloroplasts of alga *Codium fragile* and mollusc *Elysia viridis*

THE availability of inorganic phosphate (Pi) often limits plant growth, and in some cases a deficiency is avoided by the synthesis and degradation of phosphate storage compounds such as inorganic polyphosphates¹. Such compounds have been detected in yeasts², bacteria³, algae³, slime moulds³ and mosses³, but only occasionally in angiosperms^{4–7}. In algae they are synthesised in the light⁸ at the expense of ATP generated by cyclic photophosphorylation⁹, and their function as an energy reservoir and phosphate store is well established^{1,2,10,11}. The ability of algae to synthesise polyphosphate suggests that symbiotic forms may be important to the phosphate economy of their animal hosts in phosphate-deficient environments. The significance of symbiotic dinoflagellates in the 'tight' cycling of nutrients such as nitrogen and phosphorus has been suggested for coral reefs^{12,13}, although the evidence is circumstantial. We report here direct evidence of the synthesis and breakdown of polyphosphate by *Codium fragile* chloroplasts symbiotic in the ophistobranch mollusc *Elysia viridis*, which lives in the intertidal zone, where ambient levels of Pi are extremely low.

C. fragile and *E. viridis* were collected from intertidal rock pools at Bembridge, Isle of Wight, and maintained in the laboratory in aerated seawater at 9°C . Pure, intact chloroplasts were isolated from the tips of *C. fragile* fronds¹⁴, and extracted in cold 10% (w/v) trichloroacetic acid (TCA) to separate the various phosphate-containing fractions¹⁵. Two polyphosphate fractions were obtained, one soluble in cold TCA and one insoluble in acid but extractable in cold 1 M KOH. Both fractions showed acid lability¹⁵, gave a positive metachromatic reaction with toluidine blue¹⁶ and were immobile during paper chromatography¹⁷, indicating the presence of long-chain inorganic polyphosphate(s). Approximately 6% of the total phosphate in the chloroplasts was found to be polyphosphate, at levels of approximately $0.5 \mu\text{mol}$ per 10^{10} plastids, compared with $2.23 \mu\text{mol}$ per 10^{10} *Chlorella vulgaris* cells¹⁵. Thus, assuming a chloroplast diameter of $3 \mu\text{m}$, an algal cell diameter of $10 \mu\text{m}$, and that both structures are spheroid, *C. fragile* chloroplasts contain eight times more polyphosphate per unit volume

than a cell of *Chlorella vulgaris*.

To demonstrate the synthesis of polyphosphate, freshly isolated *C. fragile* chloroplasts were allowed to photo-phosphorylate for 10 min in the presence of ^{32}P i (ref. 17), followed by a chase period of light incubation before phosphate extraction and fractionation. During this prolonged incubation *in vitro* the chloroplasts were maintained in conditions optimal for carbon fixation¹⁸, and because of their extreme stability *in vitro*, remained intact throughout. The results of this 'pulse-chase' experiment (Fig. 1) clearly show ^{32}P i incorporation into the polyphosphate fraction, reaching a maximum (9.5% of the total ^{32}P i incorporated) 45 min after the removal of exogenous ^{32}P i. After 140 min of 'chase', the level of ^{32}P i-polyphosphate had dropped to 3% of the total ^{32}P i incorporated, indicating a turnover of polyphosphate *in vitro*.

These findings prompted an investigation of the incorporation of ^{32}P i into polyphosphate in freshly collected specimens of *Elysia viridis*. Of the total ^{32}P i incorporated by the slugs, 6% was incorporated into the acid-soluble polyphosphate fraction, at levels of approximately 0.1 μmol per animal, depending on the size and condition of the animals used. As an individual animal may contain between 10^7 and 10^8 chloroplasts (A.H.C. and J. Rott, unpublished data), animal polyphosphate levels may be entirely accounted for by the endosymbiotic chloroplasts.

A longer-term experiment was then designed to determine the polyphosphate content of animals stored in the dark without *Codium*. These starved animals were periodically sampled in groups of 3–5, and the resultant decline in polyphosphate levels is shown in Fig. 2. This decline suggests that the polyphosphate is slowly broken down during starvation in the dark.

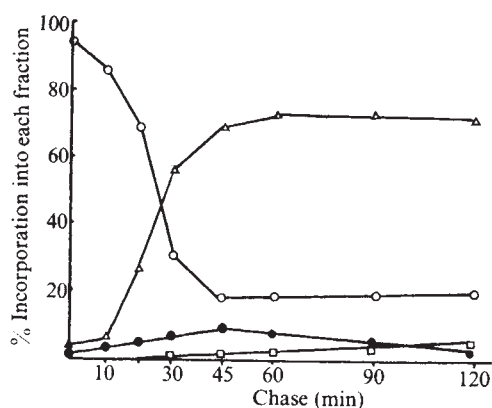


Fig. 1 The incorporation of ^{32}P i into polyphosphate by freshly isolated *Codium fragile* chloroplasts. Chloroplasts ($5 \times 10^8 \text{ ml}^{-1}$, equivalent to $40 \mu\text{g}$ chlorophyll ml^{-1}) were incubated with 5 mM MgCl_2 , 17 μM PMS, 33 μM ascorbate, 100 μM ADP and 10^3 – 10^6 c.p.m. ^{32}P i in 0.05M Tris buffer, pH 7.8, for 10 min at a light intensity of 25 W m^{-2} . They were then washed and resuspended in extraction medium¹⁴ plus carrier Pi for various periods of time. Chloroplasts were then extracted¹⁵ twice in cold 10% (w/v) TCA, and the acid-insoluble residue collected by centrifugation (2,000g). The supernatant was neutralised with M KOH, adjusted to pH 4.5 with 3M acetic acid buffer and, following the addition of a saturated solution of BaCl_2 , stored overnight at 0°C . Lipids in the acid-insoluble pellet were extracted three times with absolute alcohol and diethyl ether. The colourless nonlipid residue was then twice extracted in cold 1M KOH and the supernatant obtained from its centrifugation (4,000g) neutralised with 1M HCl, adjusted to pH 4.5 with 3M acetic acid buffer, and stored overnight at 0°C after the addition of a saturated solution of BaCl_2 . The barium polyphosphate precipitates were separated by centrifugation (4,000g), washed in 0.5M acetate buffer, pH 4.5, saturated with BaCl_2 , and with samples from all other fractions, measured for ^{32}P i incorporation by Cerenkov counting in a Searle isocap 300 scintillation counter. $\circ$, Acid-soluble polyphosphate; $\triangle$, protein phosphate and nucleic acid phosphate; $\square$, lipid phosphate; $\blacksquare$, total polyphosphate.

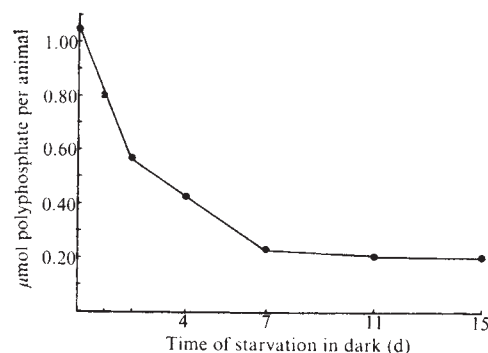


Fig. 2 The effect of starvation in the dark on the levels of polyphosphate in *Elysia viridis*. Animals were stored in the absence of alga in the dark in aerated seawater at 9°C . Groups of 3–5 animals were periodically sampled for polyphosphate content, according to Fig. 1, and were determined colorimetrically as the inorganic phosphate¹⁹ released following acid hydrolysis at 100°C .

A pyrophosphatase enzyme present within the chloroplasts is inhibited by both ATP and PGA (A.H.C. and J. Rott, unpublished data), and so is most active at times of energy demand. The situation may therefore exist whereby the endosymbiotic chloroplasts provide a reservoir of Pi and energy-rich pyrophosphate bonds in the form of long-chain polyphosphate. These accumulate in times of phosphate excess, and become available to the animal cells in conditions of phosphate demand. The presence of polyphosphate may be of importance in other symbiotic relationships, especially where there is a limited environmental supply of Pi, such as in a coral reef.

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Evidence for a C-terminal tyrosine residue in human and mouse B-lymphocyte membrane μ chains

THE antigen receptor on the plasma membrane of both human and murine bone marrow-derived (B) lymphocytes has been shown to be mainly monomeric IgM (refs 1–5), but the mechanism of attachment of this IgM is not known. Studies on other membrane proteins (for example, glycophorin⁶, cytochrome b_5 (ref. 7) and probably HLA⁸) have shown that they are bound to the membrane by means of a hydrophobic polypeptide stretch in the C-terminal

region, but secreted IgM heavy chains terminate in an extra-domain sequence of 19 amino acid residues⁹⁻¹¹, which is not sufficiently hydrophobic to act as an integral membrane component. Membrane μ chains, however, might carry a further hydrophobic C-terminal polypeptide section instead of, or in addition to, the C-terminal stretch present in secreted μ chains. To investigate this possibility, we have exploited the fact that a tyrosine residue forms the C terminus of all examined secreted μ chains, including those of human and mouse. Since it is improbable that a hydrophobic extension on the membrane μ chain would coincidentally carry a C-terminal tyrosine residue, we have examined the surface IgM from a murine B lymphocytoma, McPc 1748 (ref. 12), and a human lymphocytic B-cell line, Bristol-8 (Bri-8, obtained from G. D. Searle, High Wycombe), which also carries surface IgM (F. Kramer, personal communication), to see whether a C-terminal tyrosine residue can be cleaved from their purified μ chains by treatment with carboxypeptidase. Our data show that it can, indicating that membrane-bound IgM lacks an extra hydrophobic piece.

To provide radio-labelled μ chains for carboxypeptidase digestion, cells from each line were incubated in RPMI-1640 minus tyrosine, in the presence of L-3,5-³H tyrosine for 4 h, and then chased for 6 h in complete RPMI-1640. The cells were lysed and immune precipitates of the lysates were electrophoresed as described in the legend to Fig. 1. The gels were sliced and each slice was fragmented and incubated at 20 °C for 36 h in 10 mM phosphate, pH 7.2 (0.2 ml) containing 0.1% SDS. The radioactivity in the eluates was determined, and plotted against the gel slice number (Fig. 1). The pattern obtained for both cell lines demonstrated the presence of radioactive peaks coinciding with marker μ chains and L-chains, together with a third radioactive peak having a mobility intermediate between the two, which had an apparent molecular weight of 45,000. This material of intermediate mobility has been reported previously^{13,14}, and we have shown by peptide mapping that it is membrane actin (manuscript in preparation) which has been demonstrated to be a major component of human and pig lymphocyte membranes¹⁵.

After elution of the μ chains, the fractions indicated were pooled and each pool was mixed with myeloma IgM (3.3–10 nmol). In the case of membrane μ chains prepared from tumour line McPc 1748, mouse myeloma IgM isolated from mice carrying tumour line MOPC 104E was added, whereas in the case of membrane μ chains prepared from cell line Bri-8, human IgM isolated from the serum of a patient (Qu) with Waldenström's macroglobulinemia was added. Samples were adjusted to pH 8.0 by the addition of a 1/9 volume of 1 M Tris-HCl pH 8.0 containing 1% SDS, and then fully reduced at room temperature in 10 mM dithiothreitol. After 1 h, iodoacetamide was added to 64 mM and amidomethylation allowed to proceed for 1 h at room temperature. The samples were then dialysed for 16 h at room temperature into a solution of 1% sodium bicarbonate containing 1.6% SDS and aliquots were subsequently

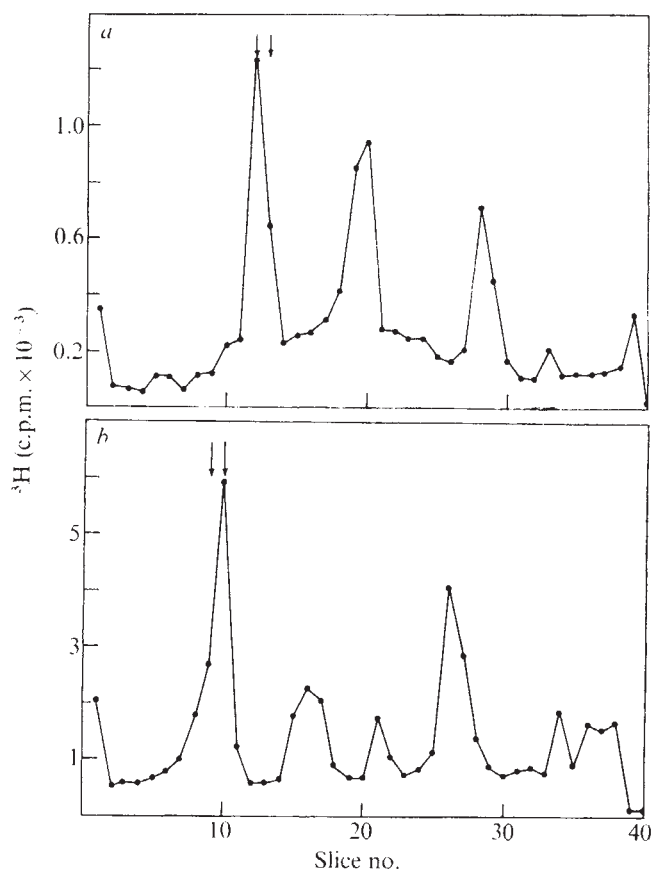


Fig. 1 SDS-polyacrylamide gel analysis of internally labelled membrane IgM from the culture of cells in L-3,5-³H-tyrosine of *a*, Bri-8 and *b*, McPc 1748. Cells (10⁸) from each line were incubated for 4 h at 37 °C in RPMI minus tyrosine (10 ml) containing ³H-tyrosine (1 mCi). The cells were washed and incubated in complete RPMI without ³H-tyrosine for a further 6 h and then lysed for 20 min at 4 °C in phosphate-buffered saline (PBS) (1.5 ml) containing 1 mM phenylmethylsulphonyl fluoride, 0.1 M iodoacetamide and 1% NP-40. The lysate was centrifuged for 30 min at 30,000g and then dialysed against four changes of PBS at 4 °C over 72 h. Aliquots of the lysate were immune precipitated for 1 h at 4 °C using 200 μ l of sheep anti-human IgM. Antigen (20 μ g) was then added to each to generate a precipitate. Precipitates were washed twice in PBS containing 1% NP-40, twice in PBS and then dissolved by incubation at 37 °C for 30 min and 100 °C for a further 2 min in 8 M urea containing 2% SDS, 10% 2-mercaptoethanol and 62.5 mM Tris-HCl pH 6.8. The solutions were then electrophoresed in 10% SDS-polyacrylamide rod gels²⁰.

digested with carboxypeptidase-A (CPA) for 30 min at 37 °C (ref. 16). As controls, equal aliquots were incubated in the absence of enzyme. After digestion, HCl was added to a concentration of 2N and the resulting precipitate was removed by centrifugation at 10,000g. The supernatant was increased to 4N in HCl and again centrifuged. Finally, the supernatant was divided into two aliquots, one of which was

Table 1 Carboxypeptidase treatment of L-3,5-³H-tyrosine radiolabelled membrane μ chains

Cell line	Secreted μ chain carrier (nmol)	Residues tyrosine released from carrier IgM by CPA digestion (nmol)	% release of tyrosine residues from carrier μ	Total radiolabel in digested sample (c.p.m.)	Radiolabel released by digestion (c.p.m.)	% release of radiolabelled tyrosine*
McPc 1748	100.0	48.0	48	19,200	370	46
	21.0	9.0	43	57,450	850	36
	37.5	19.4	52	91,040	2,020	53
Bri-8	100.0	36.0	36	34,200	920	43
	65.0	25.4	39	66,360	1,230	30

* The release of radiolabel from preparations incubated in the absence of enzyme was found never to exceed 0.4% of the total radiolabel in the digested sample.

analysed for radiolabel; the other was dried by desiccation and its amino acid content investigated using an automatic amino acid analyser. The results are presented in Table 1. According to the amino acid composition of secreted mouse μ chain¹⁷ (MOPC 104E) and human μ chain^{9,10}, assuming uniform labelling, 1/24 of the ³H-tyrosine incorporated into McPc 1748 μ chain and 1/16 of the ³H-tyrosine incorporated into Bri-8 μ chain would be present as a C-terminal tyrosine residue. Radiolabelled tyrosine was cleaved from the C terminus of both McPc 1748 membrane μ chains and Bri-8 membrane μ chains in amounts agreeing well with the yields by amino acid analysis of C-terminal tyrosine derived from the secreted μ chains used as carrier.

Our finding that both membrane and secreted μ chains have a C-terminal tyrosine residue, makes it unlikely that there is an extra C-terminal hydrophobic amino acid sequence on membrane μ chain. The absence of a hydrophobic C-terminal amino acid sequence is consistent with the demonstration that detergent binding to membrane IgM is lower than expected for an integral membrane protein¹⁸. It seems probable that membrane-bound μ chains interact strongly with an integral membrane protein, a model for such interaction being the binding of IgE to a protein in the membranes of rat mast cells and basophilic leukocytes¹⁹.

In summary, we have shown that the μ chains of the membrane IgM present on the cells of mouse B lymphocytoma McPc 1748 and human lymphocytic cell-line Bristol-8 terminate in a tyrosine residue, as do secreted μ chains.

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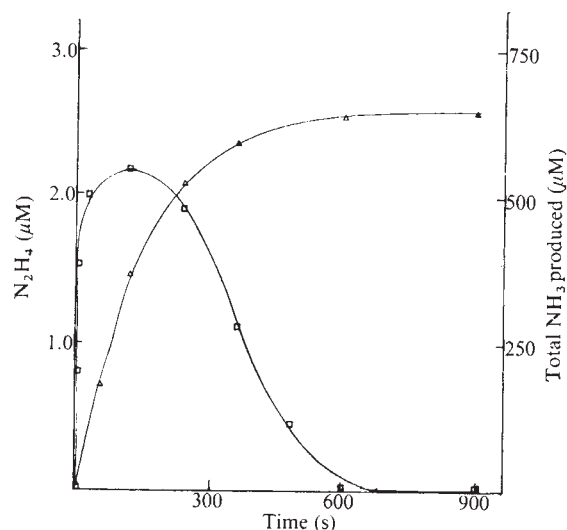


Fig. 1 Time course for concentrations of N_2H_4 (□) and NH_3 (Δ) produced during catalytic reduction of N_2 by *K. pneumoniae* nitrogenase at pH 7.4 and 30 °C. Assays (0.55 ml) contained Mo-Fe protein 20 μM, Fe protein 18 μM, $MgCl_2$ 20 mM, ATP 18 mM, creatine phosphate 18 mM, $Na_2S_2O_4$ 27 mM, creatine kinase 50 μg, and HEPES 25 mM. N_2H_4 was determined in assays quenched with 2 ml ethanol containing HCl 1 M and *para*-dimethylaminobenzaldehyde 0.07 M. After centrifuging to remove precipitated protein, the N_2H_4 concentration was determined spectrophotometrically¹⁶ at 458 nm in a 4-cm path length cell using an experimentally determined calibration curve. The points at times less than 1 min were obtained with a rapid-quench apparatus when 0.4 ml of mixed solutions were shot into 2 ml *para*-dimethylaminobenzaldehyde solution. NH_3 was determined using the method of Chaykin¹⁴.

undergo non-enzymatic hydrolysis on acid and alkali quenching of the enzyme reaction to give N_2H_4 .

Figure 1 shows the correlation between the concentrations of N_2H_4 and the NH_3 produced by highly purified nitrogenase of *Klebsiella pneumoniae*^{4,5}. The rate of NH_3 production was proportional to the concentration of the N_2H_4 -producing intermediate, and increased with a time constant of 3.0 ± 0.5 s to a maximum concentration of 2.2 ± 0.2 μM. A comparison of this time constant with the turnover time for NH_3 production is complicated by uncertainty in the concentration of enzyme reducing N_2 . In the absence of an added reducible substrate, for example, N_2 , nitrogenase will reduce protons derived from water to evolve H_2 . Saturating N_2 does not completely suppress this reaction¹. In our conditions, 70% of the electrons were utilised by concomitant H_2 evolution and 30% by NH_3 production. Assuming that only 30% of the enzyme was reducing N_2 , a turnover time of about 4 s, close to the time constant of 3 s for the appearance of the N_2H_4 -producing intermediate, can be calculated. This suggests that the rate-limiting step in NH_3 production precedes the formation of the intermediate, and this is consistent with its low maximum concentration (12% of the total Mo-Fe protein or 36% of N_2 -reducing Mo-Fe protein). After about 250 s the concentration of N_2H_4 decreased due to the accumulation of the product inhibitor MgADP. This is to be expected in our assay conditions because of the known rates of MgATP hydrolysis⁴ and the apparent K_m and K_i values for MgATP and MgADP, respectively⁶. After 600 s, when no N_2H_4 was detected, no more NH_3 was produced. The failure of the N_2H_4 to accumulate at long reaction times shows that MgADP inhibits the formation of this intermediate at least as strongly as it does its rate of removal.

In the control experiments under Ar, N_2H_4 equivalent to the maximum concentration of the intermediate (2.2 μM) was added and quantitatively recovered. This is consistent with the high apparent K_m (14 mM) for N_2H_4 calculated from the data

Biological nitrogen fixation by way of an enzyme-bound dinitrogen-hydride intermediate

THE molybdenum-containing enzyme nitrogenase¹ catalyses the ATP-dependent six-electron reduction of dinitrogen (N_2) to NH_3 . Previous attempts to detect partially reduced nitrogen intermediates, both enzyme-bound and free in solution, have failed^{2,3}. We report here the first detection of an enzyme-bound dinitrogen intermediate, which is only present when the enzyme is reducing N_2 and which on both acid and alkali hydrolysis yields hydrazine (N_2H_4). This dinitrogen intermediate need not be N_2H_4 , because the chemistry of Mo and W dinitrogen complexes (see below) suggests that other enzyme-bound dinitrogen-hydride intermediate species could

in Table 6 of ref. 7. Consequently, if N_2H_4 were a free intermediate in solution it would not be reduced by nitrogenase at a significant rate and would accumulate. As the N_2H_4 concentration decreases at long reaction times (Fig. 1), we conclude it must be derived from an enzyme-bound intermediate.

The data from times longer than 120 s in Fig. 1 were obtained from experiments in which the rate of solution of N_2 from the gas phase was such that MgATP and not N_2 became limiting after 250 s. This was not the case for the data in Fig. 2 obtained with a rapid-quench apparatus (R. C. Bray and J. Fasham, unpublished) in which the only N_2 available was that dissolved in the solution initially. In addition, H_2 , a competitive inhibitor of N_2 reduction ($K_i = 85 \mu M$)⁸, accumulates in the liquid phase to a much greater extent in the closed reaction chamber of the rapid-quench apparatus ($85 \mu M$ after about 20 s, based on the specific activity of 1,200 nmol C_2H_4 produced per min per mg Fe protein) than in the conventional assay with a 7-ml gas space above the reacting solution (about 0.1 nM after 20 s). This accounts for the more rapid decrease in the concentration of N_2H_4 in Fig. 2 (N_2 limiting, H_2 inhibited) relative to Fig. 1 (MgATP limiting, MgADP inhibited). The lower curve in Fig. 2, in which the

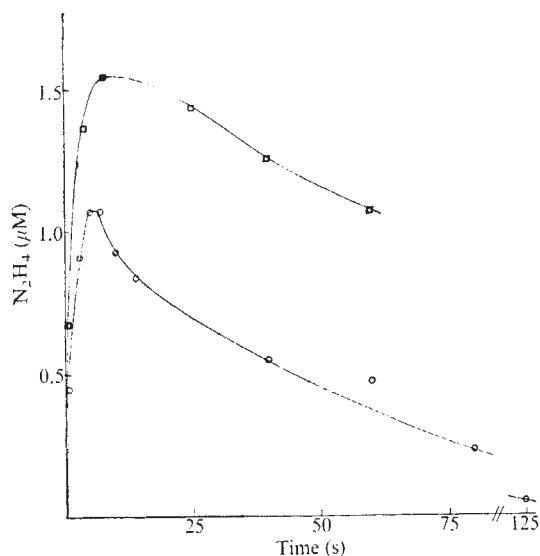


Fig. 2. Time courses for concentration of N_2H_4 obtained with the enzyme saturated with N_2 (1 atm; □) and 61% saturated with N_2 (0.08 atm; ○). Conditions and assay procedures as in Fig. 1, except that all the data points were obtained with the rapid-quench apparatus and Mo-Fe protein 17 μM and Fe protein 15 μM were used.

initial N_2 concentration after mixing was equivalent to equilibrium with 0.08 atm N_2 , shows that the maximum concentration of intermediate depends on the saturation level of the enzyme with N_2 . The observed concentration of N_2H_4 (69% of that with saturating N_2 at 1 atm) is in good agreement with the level of enzyme saturation by N_2 (61%) calculated from the apparent K_m for N_2 (0.05 atm, B. E. Smith and R. R. E., unpublished). No N_2H_4 was detected either when N_2 was replaced by Ar or when NaCN (1 mM), which is reduced to CH_4 and NH_3 , was added to assays under Ar.

Mo and W complexes containing coordinated $\equiv N \equiv N$, $\equiv N \equiv NH$, $\equiv N \equiv NH_2$, $-NH \equiv NH$ and $-NH-NH_2$ have been prepared and characterised⁹. Concerted oxidation of the metal and further protonation of the coordinated dinitrogen-hydrides give N_2H_4 and NH_3 , the relative yields depending on the reaction conditions and ligands coordinated to the metal centre^{10,11}. The structure and reactivity of these complexes together with the absolute requirement for Mo in biological nitrogen fixation¹² strongly suggest that N_2 is coordinated to Mo in nitrogenase. We have attempted to characterise the enzyme-bound intermediate by changing the

conditions in which it was detected. The same yield of N_2H_4 was obtained from the enzyme when an alkali quench (NaOH, 1 M final concentration) replaced the standard acid-quench procedure (HCl, 1 M final concentration). The alkali-quenched enzyme solution was left for 1 h before addition of the acid solution of *para*-dimethylaminobenzaldehyde used in the estimation of N_2H_4 . These data do not favour Mo- $N \equiv N$ or Mo- $NH \equiv NH$ structures for the intermediate, as the analogous W and Mo complexes in alkaline conditions give different yields of N_2 , N_2H_4 and NH_3 compared with those in acid conditions. Mo- NH_2-NH_2 or Mo- $NH_2-NH_3^+$ are unlikely structures, as our data show that N_2H_4 does not accumulate in solution. An intermediate in which the dinitrogen molecule is reduced to the -4 level with retention of some multiple bond character between the Mo and the dinitrogen-hydride ligand is more likely, that is, Mo $\equiv N \equiv NH_2$ and Mo $\equiv N \equiv NH_3^+$. These structures are consistent with the low affinity of the enzyme for N_2H_4 as a substrate⁷ and have previously been proposed¹³ as intermediates in the production of NH_3 from dinitrogen complexes of Mo and W and as models for the enzymatic reaction, whether it occurs on Mo (ref. 13) or Fe (ref. 14). Further studies on the structure and reactivity of the enzyme-bound intermediate should lead to a greater understanding of the mechanism of both biological and chemical nitrogen fixation.

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Protein folding and heterogeneity inside globular proteins

THE compact globular structure of enzymes and other proteins is the result of a struggle between two major forces. One is the entropy of the protein backbone, which strives towards an open, more random configuration, and the other is the entropy of the water environment, which drives the water-ordering nonpolar sidechains into tightly packed, water-excluding clusters. However, the protein globule would still be relatively flexible were it not for the fact that some polar groups, including those of the backbone are also found in the interior of proteins and make up a skeletal

network of hydrogen bonds. The net result is that the interior of a globular protein has continuous clusters of nonpolar sidechains separated by relatively polar regions in which nonpolar sidechains are comparatively infrequent. We have undertaken a study to quantify this structural heterogeneity and to illuminate the folding and packing of sidechains in globular proteins. This study not only uses a simple procedure for analysing protein structure, but provides a method, demonstrated here, for predicting which residues occur in the nonpolar clusters and conversely, the polar or partially polar regions of globular proteins.

To simplify the analysis, the position of each sidechain in space is described simply by the centre of mass of all sidechain atoms in the sidechain except hydrogen, the atoms all being given equal weights. Levitt and Warshel¹ have suggested representing the sidechains by single centres of action for the purpose of calculating their energy of interaction in computer simulations of protein folding. An important difference is that these workers took the centre of the sidechains as that relevant to dynamic motion of the sidechains during the folding process, and treated the sidechain as a 'dummy atom' linked to the backbone. For this analysis we make no such *a priori* assumptions: the sidechain centres correspond to those actually observed in all residues of ten proteins (refs 2-5 and personal communications from C. C. F. Blake, A. C. T. North and D. C. Phillips (lysozyme); F. Scott Matthews (cytochrome *b₅*); R. E. Dickerson (cytochrome *c*); M. G. Rossman (lactate dehydrogenase); M. F. Perutz and H. Muirhead (α and β oxyhaemoglobin)) with particularly well defined three-dimensional structures as reported.

As a first step and as a source of parameters for computer simulation of protein folding, we first analysed the geometry of attachment of sidechain centre to the polypeptide backbone, to assess the variation from type to type. Statistical analysis of sidechain geometry showed that the internal variables⁶ (virtual bond length, virtual bond angle or valence angle between bonds, and virtual torsion angle or rotation around the bond) which relate the sidechain centres to the

Fig. 1 Intramolecular distances between amino acid sidechains. Data shown here are for Leu to Ala group centres (*a*), and for Leu (*b*) and Phe (*c*) to all other group centres. The frequency data have been compensated by a radial distribution coefficient.

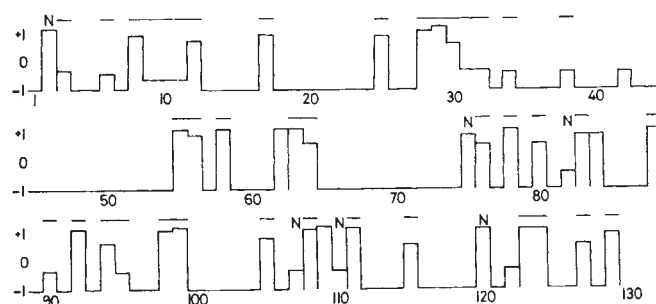
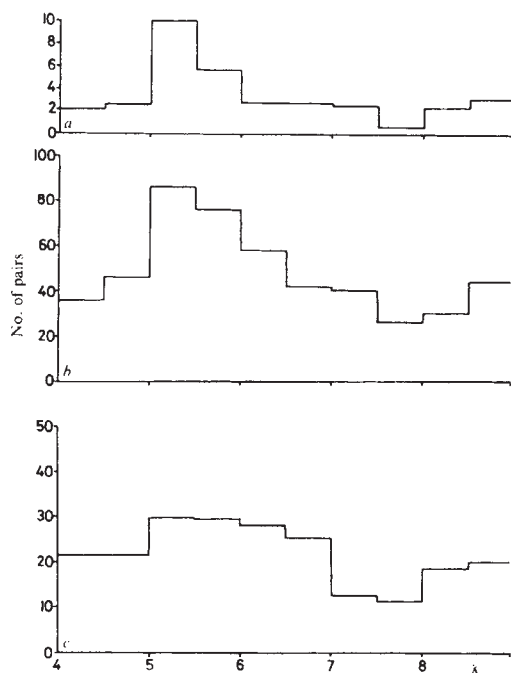


Fig. 2 The distribution of the core-forming potential expressed as an informational parameter (NATS) along the amino acid sequence of lysozyme. Residues which are defined as core by the rules in the text are indicated by bars. The letter N refers to nearby residues with insufficient contacts to be in core.

backbone of the protein have single-peaked distributions characteristic of each type (alanine, tyrosine, and so on) of sidechain. (Data are available on request.)

To summarise, we note that use of parameters based on single centres of action¹ for the more complex sidechains in computer simulation of protein folding are unrealistic except as a preliminary study. Lysine, which has one of the largest and most 'floppy' sidechains, had variations in the virtual bond length to the sidechain centre of ± 2.3 Å, in the bond angle of $\pm 52^\circ$, and of the torsion angle $C'-N-C\alpha$ -sidechain centre (measured relative to $C'-N-C\alpha-C'$) of $\pm 72^\circ$. In comparison, phenylalanine, with a nonpolar sidechain commonly occurring in nonpolar clusters, had a virtual bond length which varied by less than ± 0.6 Å, but showed variations in virtual bond and torsion angles comparable with lysine.

Next, the distances between all pairs of sidechains in the ten proteins were calculated, and the data pooled into types of interaction defined only by the types of residue involved and the distance between their centres. Assuming that the distribution of separations could be described by a radial distribution function as often applied to the analysis of liquid and solid structure, the distributions were fitted to radial distribution curves exemplified in Fig. 1.

Typically, the peak lay between 5 and 6 Å and most often at about 5.5 Å. This suggested that the shell of nearest neighbours generally occurred at this approximate distance which therefore defined sidechains in close contact. Further, the contact distances for well populated pair interactions could be approximately estimated on an arithmetic mean basis, that is, as a sum of group radii analogous to van der Waal's radii widely used in judging interatomic contacts. Although this occasionally broke down, it could be assumed to hold unless there was sufficiently strong evidence to the contrary.

In cases in which an arithmetic mean law failed, the groups were capable of hydrogen bonding to each other, or one had a cyclic sidechain and one did not. Inspection of atomic coordinates and models revealed that for potentially hydrogen bonding groups, this was because a closer contact distance involved a hydrogen bond, whereas a longer contact distance did not. For interactions involving one ring structure, this was because in the closer contact distance involved the smaller group fitting snugly into the ring, whereas the longer contact distance related to contacts at the outer ring edge.

In cases in which there was strong evidence for anisotropy, that is, for two methods of contact as for potentially hydrogen bonded pairs or for one involving a ring structure, we recorded two distances. Thus, effective group radii can be deduced from these interactions; when the pair can make a possible anisotropic contact, two effective radii are found (Table 1).

Table 1 Group radii deduced from coordinate data and frequencies of core-forming residues in the core

Residue	Ala	Cys	Ileu	Leu	Met	Phe	Try	Val
Group radii	2.0	2.9	2.9	2.9	3.0	2.2/4.2	1.4/3.7	2.5
Core	67	24	53	94	20	34	23	95
Potential core	162	38	72	130	29	53	31	125
Percentage	41	63	74	72	69	64	74	76
'NATS'	-0.352	+0.554	+1.043	+0.968	+0.830	+0.594	+1.098	+1.164
Lysozyme (actual)	7	8	6	6	2	3	5	3
(predicted)	5.0	5.0	4.4	5.8	1.4	1.9	4.4	4.6

Line 1: group radii deduced from coordinate data.

Lines 2-5: frequency of occurrence of core-forming residues in core. The results for six non-haem proteins and four haem proteins were separately analysed, found to be similar, and the combined values listed as shown. The frequencies were used to calculate an informational parameter (NATS), expressing the core-forming potential. The last two lines show the actual and predicted core composition for lysozyme.

Using the above results for sidechain contact distances, we then investigated the clustering of sidechains into non-polar and potentially polar core regions. To define quantitatively every residue as belonging to a cluster or not, criteria were developed by inspection of the contact data and molecular models. All nonpolar sidechains not normally capable of contributing to a hydrogen-bonded skeletal framework (alanine, valine, leucine, isoleucine, cysteine and cystine, methionine, phenylalanine, tryptophan) were classified as potential cluster formers.

The algorithm used will be published elsewhere and is available on request. In brief, identification of cluster residues proceeds by the computer discovering a group with about three potential cluster formers in contact, and then looking for another potential cluster former in contact with an established cluster residue. In this way, the assignment of the cluster grows progressively outwards from the initial group of about three residues. Throughout this search, sidechains on consecutive residues along the backbone were never considered to be in direct contact.

Potential cluster formers are frequently found outside clusters defined by the above algorithm. The ability to form nonpolar clusters can be analysed by similar information theory techniques to those used to measure the ability to form α -helices and other conformations⁶. This leads to a cluster-forming potential series of amino acid sidechains (Table 1). To a large extent, the distribution of residues inside and outside clusters reflects their hydrophobicity, so that the more hydrophobic a potential cluster former, the less likely it is to appear outside a cluster.

On the basis of amino acid sequence, the cluster-forming potentials of the sidechains can be used to predict sidechains occurring in nonpolar clusters. This was done by scanning successive residues in the sequence for residues with a positive cluster potential. Such a prediction for lysozyme is compared with observation in Fig. 2. The satisfactory degree of success from this simple approach suggests that the formation of large nonpolar clusters in the protein interior arises from the initial formation of small clusters belonging to relatively short sections of sequence, that is, it is strongly influenced by 'local' interactions.

To investigate the generality of this concept, as well as the usefulness of simple sidechain representations of the above type in predicting the overall conformation of a globular protein, preliminary computer simulations of protein folding from an extended conformation have been carried out using the sidechain parameters of this work. These will be reported in detail elsewhere, but three conclusions pertinent to the present work may be mentioned. First, along with formation of structures stabilised by backbone-backbone hydrogen bonding, formation of nonpolar clusters is an important first step in protein folding. Second, hydrogen bonding involving the backbone is, however, an important second step in a 'shifting' of the molecule to reach a 'native-like' structure after nonpolar clusters have been formed. And third, simplified sidechain representations, as used by us, cannot be used to predict native

conformation on the assumption that this is the only kinetically accessible structure, as a folding technique capable of negotiating barriers of about 5 kilocalories, eventually more often leads to structures which are not 'native-like'.

Good simulations probably require a more-or-less exact atom representation with realistic energy parameters and methods of treating the solvent. The adequacy of simple representations of sidechains in simulating protein folding is, in our view, not proven, in that in these earlier simulations the search was not continued for lower energy structures to which the model protein could readily escape. However, predictions exemplified by Fig. 2 are likely to provide a powerful aid in searching for the native structure of a protein of unknown conformation when a more realistic all-atom representation of the protein is used.

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reviews

Sole survivor of the great trio

J. F. Crow

Evolution and the Genetics of Populations. Vol. 3: Experimental Results and Evolutionary Deductions. By Sewall Wright. (University of Chicago Press: Chicago and London, 1977.) \$44.10; £24.50.

It is a commonplace among evolutionists, and by dint of constant repetition widely known elsewhere, that the field of population genetics was defined and largely developed by three men: J. B. S. Haldane, R. A. Fisher and Sewall Wright. Two of them wrote books in the early 1930s. Haldane's *The Causes of Evolution* was published in 1932. Although it broke few paths, it was an excellent summary of the field at that time (including Haldane's work) and was remarkable for its accuracy and lucidity. It was and still is an excellent introduction to the subject. Fisher's book, *The Genetical Theory of Natural Selection*, published in 1930, was distinguished by its mathematical inventiveness and astonishing number of novel ideas. It was almost poetic in its elegance of style and economy of words, and also in its obscurity of meaning. Characteristically, Wright did not publish a book at that time, but waited until he was more certain of his ideas and had accumulated more material. Four decades later, he has written not one book but four.

The four volumes are: Genetic and Biometric Foundations; The Theory of Gene Frequencies; Experimental Results and Evolutionary Deductions; Variation and Evolution in Nature. Volume 4 is due to appear early in 1978. Volume 3 is reviewed here. Although the four volumes are designed as a coherent, interdependent whole, one can read volume 3 with only occasional reference to its predecessors.

As the title implies, this volume is devoted to experimental studies. After a brief review of the earlier volumes, this one begins with four chapters on inbreeding and heterosis. The data come from several plants (including maize, of course), *Drosophila*, birds, guinea pigs, mice, rats, rabbits, various kinds of domestic livestock, and from humans. The kinds of traits are equally diverse: morphology, colour, yield, fertility, disease resistance, and

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many others. All the observations are compared both among themselves and with the theoretical deductions from Mendelian inheritance. Then come four chapters on selection, again with data from widely diverse sources, all analysed thoroughly and compared with theoretical expectations. One chapter is devoted to experimental studies of random gene frequency drift, many of them carried out by Wright and his coworkers or directly inspired by his theory. Another chapter is devoted to mutation and selection. Then come discussions of the mechanisms of evolution and, of course, a full description of Wright's shifting balance theory. The book ends with three chapters devoted to genetic load, to the evolution of dominance, and to a discussion of breeds of livestock.

The diversity and enormity of the literature surveyed are impressive. But far more impressive is what Wright does with it. The various studies are not simply reviewed: they are interpreted, compared with others, put into a common framework, and related to the general question of evolutionary mechanisms. Often the data are completely re-analysed, sometimes with interpretations that are far richer than those of the original authors. Wright's capacity to find interest and relevance in work that many would regard as uninteresting, unpromising, *passé* or too complicated, is everywhere evident. So is his respect for data and experimental findings; all are taken seriously, and none glossed over if they do not

fit his notions. The analysis is always fair, complete, and meticulous. His generosity toward other authors is ever apparent.

Wright is always interested in the biological relevance of the theory, and this means taking all pertinent factors into account. It also means that there cannot be a high level of precision. In this, he is in contrast to both Fisher's mathematical elegance and finesse and to the trend of many modern mathematicians who formulate highly specific, but limited models. He is first and last a biologist, and his mathematical analysis is always closely tied to his picture of biological reality. Nowhere is that more evident than in this volume, in which theory and data are constantly brought together.

Wright's classic studies on guinea pigs are especially well summarised. The book also contains something unusual in recent discussions of evolution: a review of the history of several breeds of livestock, especially shorthorn cattle. Wright genuinely enjoys guinea pigs and fine livestock and this affection becomes apparent to the reader. His ideas on evolution were first formulated while he was employed as animal husbandryman by the US Department of Agriculture in the 1920s. He was impressed by the ubiquity of gene interactions and pleiotropy in his guinea pig studies. He also noticed that improvement of breeds of livestock came about by the development of high excellence in a few herds, partly by random processes, and the diffusion of this high quality through the breed from the use of sires out of the best herds, rather than by mass selection in the whole breed. Here are the origins of his evolutionary theory, whereby a structured population permits shifts from one harmonious gene combination to another under the influence of random differentiation, selection within and between demes, and migration.

Much of Wright's work, both experimental and theoretical, is reviewed in this book (and in the other volumes). This is often better for the reader than the original papers. For one thing, Wright has written an enormous amount, and it is convenient to have it summarised. For another, the newer, more general formulations are usually easier to understand than his earlier

Photo: Lewellyn Studio, Chicago

Sewall Wright

writings. But most important, here we have access to Wright's most mature thoughts. It is nevertheless remarkable how consistent has been his view of the evolutionary process from 1930 until the present.

Of course, most of the book reports the work of others. There are about 700 references in the bibliography. Many of these writings will not be familiar to readers, for Wright has been indefatigable in searching out relevant literature from diverse sources. Many unnoticed or forgotten papers are given a new significance. Another indication of the wide coverage is the number of authors referred to. There are about 500 names listed in the

author index (not including his own, which Wright chose to omit).

Wright is the sole survivor of the great trio. He has spent almost all his time for more than 15 years on these books. When the man who has contributed more to a field than any other living person takes time to review the whole field and put it into a common framework with his own synthesis, we should anticipate something important. Having seen volume 4 in manuscript, I know that this anticipation will be fully realised. □

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But is it ecology?

Nature's Economy. By Donald Worster. Pp. xii+404. (Sierra Club Books: San Francisco, 1977.) \$15.

It helps a reviewer when an author explains what his book is supposed to be about. The publisher's blurb, on the dustcover, may be misleading, and only say what the publisher hopes the author has written. Donald Worster, in his preface, states that "the aim of this book is not so much to account for the appeal of ecology to our own times as to understand what this field of study has been prior to its most recent ascent to oracular power". The trouble is that though it is indeed about the way in which people have thought about nature and their surroundings during the past 200 years, much of this thought has very little to do with what I would call ecology.

The word ecology means many different things to different people. I have given this subject some thought, particularly since I had the task of drafting a definition for the new Fontana *Dictionary of Modern Thought*. Here, with due acknowledgement to Ernst Haeckel (who is believed to have coined the word in 1873) I say that it is "the branch of biology which deals with the interrelationships of organisms with their environment". I briefly sketch the subject's history, mentioning the main subdivisions of ecological study. I take the opportunity of riding one of my hobby-horses, and, while praising the 'muddy-boot' ecologist, slip in a snide comment about those with a more theoretical approach. Finally, I deplore the misuse of the term today, when it is used to cover anything considered good, so that "the ecology of litter" comes to mean voluntary garbage collecting by public-spirited college students. I con-

clude my contribution by saying: "because of these debased uses, it seems likely that scientific ecologists will, in the future, have to find some new term to describe their activities".

I must confess that I had some misgiving when I read Worster's comment about ecology's alleged oracular power. However, he hardly develops this point, and says very little about the current period other than describing it as the "Age of Ecology". In fact, rightly in my opinion, he concludes that the modern doomwatchers owe very little to the scientists and others who have studied the subject, and the environment, during the past 200 years.

Worster's technique is to describe the life, work and thoughts of the men whom he thinks have made important contributions to his subject. He starts with Gilbert White (1720-1793), the English country parson and the author of *The Natural History of Selborne*. He includes White's contemporary, Linnaeus (1707-1793). He contrasts White's arcadian idyll with Linnaeus' more scientific approach. He then devotes several chapters to Thoreau (1817-1862), described as a subversive, in contrast to Charles Darwin (1809-1882), who is credited with inaugurating a dismal age, the idea of the survival of the fittest being seen as suggesting that every organism strives to do down the rest. These four men are the subject of rather more than half the text, although they all worked before 1873, the year when Haeckel started using the word ecology. Worster's accounts of their ideas are well expressed, though they could with advantage be shorter and less dramatically written. But the conflicts of opinion which he deems to be so significant are, in the long run, much less important than the way in which these men collected and codified accurate information about the flora and fauna of the world in which they lived. Many of their philosophical observations had little

effect on the progress of science.

The remainder of the book deals, much more briefly, with ecologists from the late nineteenth century to the present day. Those considered in most detail are Eugenius Warming, Frederick Clements, Aldo Leopold, Arthur Tansley and members of the so-called New Ecology Group, which arose in Chicago in the 1930s, and which led to the recognition of the importance of energy flow and eco-economics.

Although the contribution of these workers is described satisfactorily, Donald Worster gives a very curious picture of the development of ecology. We are given the impression of warring factions, men wedded irrevocably to their pet theories, and, when defeated, retiring (with their ideas) to a scientific limbo. We are told that by the 1930s, the ideas of Darwin were considered "not so much wrong as outmoded and boring", and that by the 1960s "orthodox scientific thought was virtually monopolised by thermodynamics and bioeconomics". As one who lived, and sometimes worked as an ecologist, during that period, I cannot recognise this picture.

Real ecology has always been, and continues to be, a descriptive science. Ecologists wish to know as much as possible about the animals and plants which they study, and their relationships with each other and their environment. The different approaches are complementary to each other, not warring factions desiring each other's destruction.

Finally, I fear that the "oracular power" of the ecologist is a myth. Actually, Donald Worster does much to explode it. He shows that the doomwatchers of today owe little to the scientific work of present-day or of past ecologists. He suggests that they choose suitable bits of theories of all past generations from Gilbert White to today, and that they modify them for their own purposes. I think they are less well informed, and that most of them rely more on their feelings than on their knowledge or their powers of observation. Unfortunately, they are joined by some otherwise-reputable scientists who do not always make it clear when they are speaking as experts in their own subject, and when as concerned citizens with no real expertise. They mean well, but they may bring science in general, and ecology in particular, into disrepute. This is why we may need a new name for "that branch of biology which deals with the interrelationships of organisms with their environment".

Kenneth Mellanby

Kenneth Mellanby was Director of Monks Wood Experimental Station from 1960-74.

Abnormal haemoglobins

Human Hemoglobins. Edited by H. F. Bunn, B. G. Forget and H. M. Ranney. Pp. 432. (Holt-Saunders: London; Saunders: Philadelphia, 1977.) £17; \$18.

STUDIES of the inherited disorders of haemoglobin have contributed greatly to knowledge about abnormal gene action in human disease. In addition, they have made a considerable impact on such diverse fields as population genetics, anthropology, and developmental biology. The subject came into its own in 1949 with the discovery by Pauling and his colleagues that patients with sickle cell anaemia have an abnormal haemoglobin. Since then much has been learnt about the genetic control and synthesis of the human haemoglobins, and in the past few years the genetic defects in several disorders of haemoglobin synthesis have been defined at the molecular level. Indeed, the human haemoglobin field is one of the few areas in which molecular biology has made a major impact on clinical medicine.

It was realised in the early 1950s that there are two main types of inherited disorders of haemoglobin. First, there are the structural haemoglobin variants which usually result from single amino acid substitutions in one or other of the globin chains. Although many of these are harmless, some cause a clinical disorder by altering the configuration, stability or function of the haemoglobin molecule. In addition to helping clinicians to understand the pathophysiology of some common inherited anaemias, investigation of these haemoglobin variants has provided valuable information about the relationship between the structure of haemoglobin and its function as an oxygen carrier.

Even more fascinating is the information which has been obtained from researches into the disorders characterised by a reduced rate of synthesis of the globin chains of haemoglobin, the thalassaemias. These conditions occur commonly throughout the Mediterranean region, the Middle East, India and Pakistan, and South-East Asia. They cause a major public health problem in these countries and are among the commonest single gene disorders in man. Analysis of the molecular pathology of the thalassaemias has yielded a remarkable diversity of causes for the reduced rate of haemoglobin synthesis. These include gene deletions, chain termination mutations, abnormal crossing over with the production of fusion genes, frame shift mutations, abnormalities of gene transcription, and the production of structurally abnormal mRNA. Studies of the related disorder, hereditary persistence

of fetal haemoglobin, have shown that areas of the cluster of haemoglobin genes are involved in the suppression of fetal haemoglobin production in adult life. Further investigation of these disorders by the recently developed techniques of restriction mapping and structural analysis of the globin genes promises to open up another fascinating chapter in the human haemoglobin field within the next few years.

The authors of *Human Hemoglobins* are well qualified to review this exciting field. Helen Ranney was first to show allelism between the genes for haemoglobin S and haemoglobin C and has made many important contributions to our understanding of the pathophysiology of the abnormal haemoglobin disorders. Frank Bunn has contributed a great deal to knowledge about the function of both normal and abnormal haemoglobins, and Bernard Forget has done valuable work in unravelling the molecular pathology of the thalassaemias and on the structure of human mRNA. As might be expected this trio have produced an excellent book. Early chapters deal with the structure and function of haemoglobin and the way this is modified in various disease states. Subsequent sections deal with the structural haemoglobin variants and thalassaemias, and the book ends with an excellent section describing some of the acquired disorders of haemoglobin. The writing is clear, lively and remarkably uniform for a multi-author work. The book is very well produced, remarkably

free from errors of fact or type, and well illustrated; and there is an extensive and up-to-date bibliography. By current standards, it is reasonably priced.

It is impossible for one modest sized volume to deal comprehensively with every aspect of the abnormal haemoglobin field. If this book has a fault it is in its rather superficial treatment of the more clinical aspects of the structural haemoglobin variants and thalassaemias. Furthermore, it makes no attempt to deal in detail with such problems as genetic counselling, the population genetics of the haemoglobin disorders or the more practical aspects of laboratory diagnosis of these conditions. However, these minor criticisms detract little from what is an excellent account of a fascinating field. Indeed, the sections on the function of haemoglobin and its modification in different diseases, which are by far the clearest descriptions of this complicated subject that the present reviewer has read, alone are worth the price of the book. Both authors and publishers should be congratulated on *Human Hemoglobins*. It can be thoroughly recommended to anybody who wants an up-to-date account of this rapidly developing field and should be compulsory reading for those who believe that molecular biology has made no contribution to clinical practice.

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Mammalian circulatory mechanics

The Mechanics of the Circulation. By C. G. Caro, T. J. Pedley, R. C. Schroter and W. A. Seed. Pp. 527. (Oxford University Press: Oxford, London, New York and Toronto, 1977.) £22.

ABOUT one third of this book deals with basic mechanics, and as might be expected the emphasis falls strongly on the fluid dynamics. Personally I would have liked rather more on solid mechanics and mass transfer, but the balance is not at all unreasonable and the material is presented with admirable clarity. In the remainder, mammalian circulatory mechanics are discussed at length. There are chapters on the heart and the veins, regions frequently covered scantily (if at all) in texts of this sort. I was least satisfied with the chapter on the heart, but this really reflects the extreme difficulty of describing its action both succinctly and accurately. Most of the biological chapters contain suggestions for further reading, but the physical ones do not.

The book reads well and gives little sign

of having been written by four authors, two with a medical background and two physical scientists. Although the quirky individuality of McDonald's *Blood Flow in Arteries* (its major competitor) is missing, this new work covers more ground and in greater depth, and deserves to become a standard text. Much has been learnt in this field over the past 20 years, and further progress is likely to be slower and tougher. This is a good time therefore, to take stock and those who want to know what has been achieved will find here a good summary. Although the authors address their text principally to biologists and physicians, my own feeling is that it might be of rather greater interest to those approaching the subject from the other side. Although not intended as a research text, I would have welcomed some references and more suggestions for further reading, especially in the first part. The book is well produced, the illustrations are clear and to the point, but at £22 few will be able to afford their own copy; however, I would want to place it in undergraduate and hospital libraries.

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Influenza vaccine campaign

Influenza in America, 1918-1976: History, Science and Politics. Edited by June Osborn. Pp. 135. (Neale Watson: New York, 1977.) \$7.95.

THE deceptively simple title of this book conceals the drama of America's 1976 swine influenza vaccine campaign, the account of which will interest many not directly concerned with the influenza problem. The story begins with a brief account of influenza in 1918 in the USA written by the historian Alfred Crosby, and ends with the two National Immunisation Conferences held to examine the implications of immunisation policy in its broadest sense, following the 'death' of the swine 'flu' campaign in December 1976. In the final act of the campaign, the surveillance following the mass vaccination had revealed the occurrence of neurological complications in one in 100,000 persons among the 40 million who received the vaccine. Thus, the prolonged debate in Congress and Senate described by Arthur Visellear of Yale University, which followed the launch of the campaign by the President, stands fully justified. The insistence of those who manufactured the vaccine upon indemnification from liability for vaccine complications was the consequence of an inability of Insurance Companies to underwrite such a problematical risk and thus involved Congress as well as the Government. So it was in 1976 that an almost chance discovery in the laboratory led step-by-step to a problem of preventive medicine involving Government, health workers and millions of ordinary men and women in an effort to ward off a threat, which never materialised.

Professor June Osborn of the University of Wisconsin, who contributes jointly with Dr Donald Millar the preface to the account of campaign and solely the epilogue, is to be congratulated on providing such a clear account of the issues which involved the whole American nation and also Canada, and which, in spite of the author's euphemism of an overall gain, caused enormous public repercussions and reactions against far more than immunisation for influenza.

To recapitulate, the background to the decision early in 1976 began with the recovery of a virus (A/New Jersey/76) from soldiers with influenza in a military camp at Fort Dix. The virus bore antigens apparently the same as those of the swine influenza virus recovered by the late Richard Shope in 1931 and still a cause of endemic swine influenza in the Middle West. Because of the general acceptance of Shope's virus as the survivor of the 1918 human pandemic virus, the New Jersey discovery immediately led to the supposition that a new pan-

demic of 1918-type was threatened. In all that followed, the impression given in this monograph is that fear of this possibility dominated the virologists' advice and motivated the Presidential Conference that something must be done in this 'the Bicentennial year' to combat the threat to the US citizens. Alas, the strange lack of transmission of the disease due to the New Jersey virus outside the walls of the camp was not allowed to interfere with the decision to vaccinate, which, through shortage of time for manufacture, was allowed to proceed. Thus was the historical decision made and it seems clear in retrospect that the ghost of 1918 had forced the virologists' opinion against their better judgement.

Those interested in immunisation will find the account of the legislative debate given in an Appendix of great medico-legal interest. For British readers there is an analogy with the involvement of the House of Commons in the controversy over immunisation with whooping cough vaccine, though in the UK the issue in 1977 was one of compensation rather than of litigation. Yet, as was so wisely pointed out during the debates in Congress concerning liability, the solution by indemnity provided a hazardous prece-

dent, and the account now being presented by sufferers from the post-vaccinal Guillain-Barré syndrome, could be of astronomical proportions.

Does this book serve to point the way concerning future policy for immunisation against influenza? The answer seems to be no, for new pandemics will arise in which the harkback to 1918 influenza is absent. Indeed, the dilemma has already presented itself in the shape of the 'Russian' A1 virus, which originated in China and which is an apparent resurgence in 1977 of the virus familiar to influenza workers in the decade 1946-1957. One lesson is clear—the public has the right to be involved at an early stage in any decision concerning millions of its members and described picturesquely as go-no go. Health education, at least on immunisation matters, has, it seems, a long hard road to travel. It is only a perceived risk from disease and not a probability which seems likely to serve to restore public confidence in the judgement and advice of microbiologists.

Charles Stuart-Harris

Sir Charles H. Stuart-Harris is Chairman of the MRC Committee on Influenza and other Respiratory Virus Vaccines.

Solute movement in the soil-root system

Solute Movement in the Soil-Root System. By P. H. Nye and P. B. Tinker. Pp. 542. (Blackwell Scientific: Oxford, 1977.) £12.80.

THIS important book is the first to deal comprehensively with solute movement in the soil-root system. The subject matter has only come into prominence during the past 10 years, during which time researchers have begun to investigate the kinetics of solute flow in soil resulting from solute absorption by roots. Much research on soil-plant relationships has been of an empirical nature or has measured static systems, because of the lack of understanding of the dynamic processes operating in the complex root-soil system. This organisation of recent research on solute movement in the soil-root system and its clear presentation in this book should stimulate awareness as well as research activity in this area.

Though not a large book, it provides a good coverage of the subject. The authors have organised the subject matter into eight chapters. After presenting recent history and the approach they use in writing the book in the first chapter, they discuss water flow in the second. Water flow in the soil either by gravity or as a consequence of plant root absorption is one of the mechanisms of solute transport within the soil. They then discuss solute interchange between the solid,

liquid and gas phases in the soil. This interchange governs solute concentration in solution. They treat both organic and inorganic substances which is particularly important because of the increased use of organic pesticides and the significance of their movement in soil. The principles of movement of solutes in the soil by mass-flow and diffusion are elaborated in a chapter on local movements. The chapter on solute uptake by the plant root is confined mainly to significant properties of plant roots that influence the kinetics of solute influx into the plant root.

At this point in the book the authors are in position to combine the transport processes in the soil with the absorption properties of the root and they call on much of their own research in doing so. In addition to discussing the theory and measurement of solute concentrations profiles around plant roots, the authors include a discussion on the physical, chemical, and biological effects of roots on the rhizosphere soil. Moving from the simpler system to the more complex community of plants, they organise the information using models for simulating solute uptake and growth of the plant.

I recommend the book as a reference for teachers and for research workers in soils, plant nutrition and biology, and also as a text for courses on soil-plant relationships. The long list of references at the end of the book is particularly useful.

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Recent scientific and technical books

Technology

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obituary

William Klyne

PROFESSOR William ('Bill') Klyne died on 13 November 1977, at the age of 64. Tragically his death came only six weeks after an early retirement from the Chair of Chemistry in Westfield College, University of London. With characteristic courage and determination in the face of declining health, he had resolved to shed the burdens of administrative office in order to devote his time as fully as possible to the continued service of chemistry. To this end, he had recently been awarded a Leverhulme Emeritus Fellowship, and intended to visit his many professional friends in Europe to gather material for a new book on circular dichroism, his major research interest in the last twenty years of his life.

Bill Klyne was born on 23 March 1913, in Enfield, Middlesex. He was educated at Highgate School and New College, Oxford, where he felt privileged to have worked for a year with the late Sir Robert Robinson.

In 1936 he was appointed Assistant in Medicinal Chemistry at the University of Edinburgh, under Professor Guy Marrian. A thorough reorganisation of the course of chemistry for medical students led him to write his first book *Practical Chemistry for Medical Students*. One of the students who assisted him with the proofs was Barbara Clayton, who later became his wife and is now Professor of Chemical Pathology at the Institute of Child Health, University of London.

In 1947, after completing a Ph.D. degree at Edinburgh, he moved to the Postgraduate Medical School at Hammersmith Hospital in the University of London, where he was first a Lecturer and later a Reader in Biochemistry. His main research activity at Hammersmith comprised a series of studies into urinary steroids, from both the biochemical and the chemical point of view. The amalgamation of these two disciplines was a feature which continued throughout his life. It brought him into contact with persons in areas ranging from medicine to chemistry, and enabled him by 1954 to obtain the support of the Medical Research Council for the establishment of its Steroid Reference Collection, of which he later became Curator. His well-known monograph, *The Chemistry of the Steroids* was published in 1957 and has since been translated into both French and Spanish.

Also while at the Postgraduate Medical School, he was among the pioneers in the application of flame photometry for clinical purposes. This work was done in collaboration with a Dutch scientist, Dr. W. R. Domingo, an early example of Bill Klyne's special gift for forging links with overseas workers.

During the 1950s, as a timely development from the use of optical rotation differences as an aid to the study of molecular structures, Bill Klyne became involved in the early application of optical rotatory dispersion to organic chemistry, and installed one of the first commercial spectropolarimeters in his laboratory. In later years he frequently recalled a visit to the USA in 1958, where, with Moffitt, Woodward, Moscovitz, and Djerassi, the now familiar 'Octant Rule' was conceived as the first and still the most useful basis for the correlation of chiroptical data with molecular structure. His interest in stereochemistry also led him to edit or contribute to the four volumes of *Progress in Stereochemistry* (1954-69).

Bill Klyne was appointed in October 1960 as the first Professor of Chemistry at Westfield College, in the University of London. As one of the small group entrusted with the founding of the Science Faculty in a college which had previously concentrated on the arts, he built up the new Department of Chemistry, combining undergraduate teaching with the establishment of a research team which, despite its small size, gained international recognition in his chosen areas of study.

He took with him to Westfield College the MRC Steroid Reference Collection, which by this time had become known world wide, and was distributing many hundreds of samples of rare steroids annually to clinical and biochemical laboratories in over forty countries. In acknowledgement of the value of the collection to medical science many of the world's most famous workers in the steroid field have donated samples from their own stocks. The United States National Institutes of Health have also supported the work since 1965.

Professor Klyne's other area of research, into chiroptical phenomena, expanded rapidly after his arrival at Westfield College. For the past 20 years his research group has provided a service in circular dichroism, funded since 1962 by the Science Research

Council, and has given formal assistance in this specialised field to many chemists overseas. Chiroptical phenomena provided a fruitful field of research which led to the publication of nearly 100 papers in chemical journals. His *Atlas of Stereochemical Correlations* published in 1974 with Dr. J. Buckingham, received wide acclaim.

Professor Klyne's integrity and profound concern for the affairs of Westfield College was recognised by his appointment to a number of influential posts in the college, including Dean of Science (1971-73) and Vice-Principal (1973-76). Outside the duties of college, department, and research, Bill Klyne was widely known and greatly respected for his contributions to the wider service of chemistry. He devoted a great amount of time to the Chemical Society, and was one of its honorary secretaries from 1966-72; he later served for two years as a Vice-President of its Perkins Division. He was a member of the Biochemical Society and served on its Editorial Board from 1950-55. Within the University of London he played a major part in securing the agreement of other Chemistry Departments to participate in an intercollege research scheme (ULIRS) for the sharing of expensive research facilities, now a thriving model of co-operative planning. His international reputation grew further with membership of the IUB-IUPAC Commission on Biochemical Nomenclature (1958-77, including six years as Chairman) and of the IUPAC Commission on Nomenclature of Organic Chemistry (from 1971). He served the IUPAC on a number of other Committees, and shortly before his death had been appointed Chairman of the new Sub-Committee on Chiroptical Phenomena.

Bill Klyne always enjoyed those occasions when chemistry took him overseas, giving him opportunities to meet his many friends in other countries. Visits to Switzerland gave him particular pleasure. Although he allowed himself little time for complete relaxation he delighted in going with his family, and 'Bumpy' the labrador, to their holiday home in Dorset. He took real pleasure in many small or familiar things, like the arrival of stamps from colleagues overseas for his collection, the large number of maps accumulated during his travels, the sending and receiving of postcards and Christmas cards, and in showing visitors

the view over London from Westfield College.

Those who knew Bill Klyne, whether professionally or personally, felt a strong bond of affection, coupled with admiration and respect for his strength of character and his integrity. He showed deep concern for others, and particularly for his colleagues and their families, many of whom have reason to be grateful for his help or sound advice in resolving personal or professional problems. His kindness was invariably combined with a great sense of fairness and propriety: always meticulous in his dealings with friends and associates, he expected high standards of them, but none more rigorous than those he demanded of himself.

He leaves his wife, Barbara, their son Michael, and daughter Janey.

D. N. Kirk
P. M. Scopes

L. F. Bates

LESLIE FLEETWOOD BATES died peacefully on 20 January 1978, after a short illness, at Nottingham, the city where he had made his home for over forty years. With his death the study of magnetism in Great Britain has lost one of its most distinguished elder statesmen and its most passionate advocate.

He was born in March, 1897 at Kingswood, Bristol where he acquired that curious habit of speech in which the letter "l" is added to the end of words normally terminated by a vowel. The word which suffered most in this respect was "idea" and many of his research students were at some time puzzled by his enthusiastic description of what always sounded like "a good ideal for research".

Bates entered the University of Bristol in 1913 at the remarkably early age of 16. After graduation he immediately enlisted in the army and after a short period was sent to India where he was placed in charge of the X-ray laboratory of the 9th division with the rank of Captain.

In 1920 he returned to the University as a post-graduate student and began research in magnetism under the supervision of A. P. Chattock. He was required to determine the gyro-magnetic ratio or Landé g -factor for ferromagnetic metals by a direct measurement of the angular impulse acquired by a ferromagnetic rod when it is magnetised. It was found, in all cases, that g was close to 2. For nearly fifty years it has been fashionable to present this result as evidence for the spin-only nature of ferromagnetism in iron and nickel. However, in an address to the Arbeitsgemeinschaft

Ferromagnetismus some fifty years later Bates reminded his audience that, at the time, the results were regarded as much needed evidence for the very existence of electron spin.

The records of the University of Bristol reveal that in 1921 "the Science Board recommended that Mr. L. F. Bates be awarded the British Association Exhibition for the current year." It is not known whether he actually took up the exhibition since he left Bristol in 1922 for Cambridge. There, in the Cavendish Laboratory he undertook research concerned with long-range alpha particles under the supervision of Lord Rutherford. Bates was very proud of his association with Rutherford, but it is difficult to avoid the impression that neither of them was inspired by the topic and when Bates left Cambridge for a lectureship at University College, London, it was to magnetism that he turned his attention.

He engaged in studies on permanent magnets using the magnetic potentiometer, that invention of Chattock's which fascinated Bates all his life; magnetic susceptibility studies on amalgams and, very much in advance of the time, the magnetic properties of compounds of manganese. He prepared MnAs and MnSb and, in later life, bitterly regretted having been diverted from MnBi when, in the 1950's, that compound looked like becoming the permanent magnet material of the future.

In 1936 he was appointed Lancashire-Spencer Professor of Physics at University College, Nottingham and for some thirty years Nottingham became almost synonymous with magnetism. With E. C. Stoner at Leeds and W. Sucksmith at Sheffield expertise in magnetism became so heavily concentrated in the three universities that the region was often referred to as the "magnetic belt". At Nottingham Bates embarked on his elegant and successful method for measuring temperature changes during the hysteresis cycle of ferromagnets and, during the war, acted as adviser to the Inter-Services Research Bureau where he was particularly concerned with the degaussing of ships.

Towards the end of the war he persuaded the Electrical Research Association to support some work on magnetic domain (Bitter) patterns. At a distance of thirty years this looks like a logical complement to the work on the heat of hysteresis. In fact it is an example of Bates' highly intuitive approach to physics. The current state of Bitter patterns at the time is admirably summed up in four photographs reprinted in Becker and Döring's *Ferromagnetismus* which had appeared in 1939. They present a picture of be-

wildering complexity and give no indication whatsoever of the powerful tool the technique was to become. The breakthrough was made in the U.S.A. but when it came Bates and his students were poised to exploit it and immediately provided a dramatic experimental verification of a remarkable and unexpected prediction by Néel that domain sizes in crystals of certain shapes would actually shrink in the presence of an external field.

University College, Nottingham, became Nottingham University in 1948. Bates, who had always played an active role in university affairs found himself in demand at all levels of university life. He served on all its important committees and was Deputy Vice-Chancellor from 1953 to 1956. After retirement in 1964 he remained very active. He was very proud of his association with the University of London and was only too pleased to retain this association through a long period as one of its external examiners. He was Chairman of the Royal Society's Symbols Committee and was responsible for producing its most recent publication *Quantities, Units and Symbols*. But magnetism remained his greatest love. He attended all the important international conferences, his last appearance being at ICM-76, Amsterdam. Until last year, when ill-health restricted his mobility, he was to be seen at every meeting of the Institute of Physics' Magnetism Group, usually in conversation with one of his former students in whose work and welfare he took a genuine and often fatherly interest.

As a man he had a transparent and child-like honesty that left no room for subterfuge or intrigue. He was never afraid to speak his mind and those who knew only his gentle demeanour were often taken aback by his forthright and often wrathful denunciation of any underhand manoeuvre or self-seeking enterprise.

By nature gregarious, his very active social life was somewhat curtailed after the death of his wife shortly after his own retirement and there were times when he must have been a very lonely man. In later years he derived great pleasure from his membership of the Athenaeum where he frequently entertained his former colleagues and students to dinner. On these occasions the years would roll away, the boisterous good humour and high spirits of his former glory would return; and as the conversation ebbed and flowed a chance remark would reveal the remarkable insight and compassion of the man whom we shall always remember with admiration and affection.

E. W. Lee